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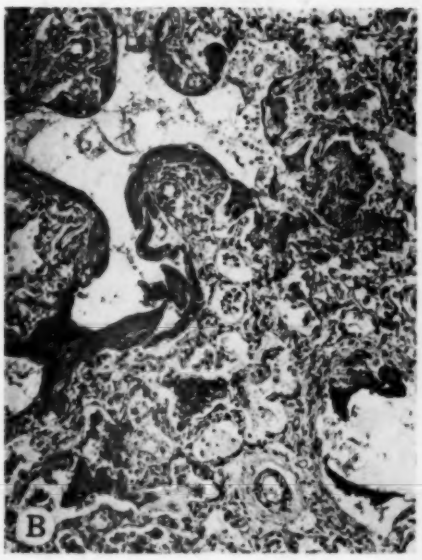

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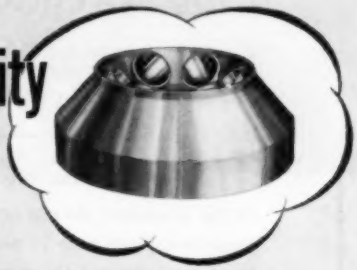

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A.M.A. Archives OF PATHOLOGY

Castration Changes in the Pituitary with Seminiferous Tubule Atrophy

HUGH V. BELL, M.D.
and
ABNER GOLDEN, M.D., Emory University, Ga.

Castration of the experimental animal is associated with characteristic and apparently specific cytologic alterations in the adenohypophysis.* These consist of a marked numerical increase and vacuolation of basophile cells and the presence of many swollen chromophobe cells with basophilic cytoplasmic stippling. There have been rare reports of similar findings in the adenohypophysis of human castrates.† We recently had the opportunity to study a patient with seminiferous tubule atrophy and Leydig-cell hyperplasia in whom striking changes were observed in the adenohypophysis. These findings are of particular interest in that they shed some light on the nature of the pituitary changes in castration and on the problem of the sites of hormone production in the human testis.

REPORT OF A CASE ‡

D. M., a 66-year-old white man, was admitted to Emory University Hospital Nov. 21, 1954, with signs and symptoms of cerebral hemorrhage. Diagnosis of bronchial asthma, cor pulmonale, and a high interventricular septal defect had been established during previous hospital admissions. Medication had included penicillin, streptomycin, aminophylline, digitalis preparations, and mercurial diuretics. He was given 105 mg. of corticotropin intramuscularly over a nine-day period in October, 1953, but had received no other hormone therapy.

Sexual history revealed normal marital relations until 10 years before admission, when his pulmonary symptoms became disabling. He had been married twice but had no children. His first wife underwent hysterectomy because of a uterine cancer, and his second wife had had a hysterectomy prior to marriage. No history of orchitis or testicular injury was obtainable from the patient's records.

Physical examination revealed a left hemiplegia. The blood pressure was 130 mm. Hg systolic and 70 mm. diastolic. The lungs contained numerous rhonchi and wheezes. The heart was enlarged, and a rough Grade 3 systolic murmur was heard over the base and apex. The external genitalia were normally developed. The hair was of coarse texture and of normal male distribution.

Admission laboratory studies were noncontributory. White blood cell count, differential count, and blood hemoglobin content were within normal limits. There was no glycosuria.

The patient rapidly became comatose and died 26 hours after admission.

POSTMORTEM FINDINGS

Autopsy was performed two hours after death. The body was that of a well-developed and moderately well-nourished white man. Distribution of hair and adipose tissue was normal. There were no abdominal striae. No abnormalities of the breasts were noted, and

Submitted for publication May 9, 1955.

From the Department of Pathology, Emory University Hospital, and Emory University School of Medicine.

* References 1 through 3.

† References 4 and 5.

‡ Dr. E. C. Cunningham, Decatur, Ga., gave permission to use his records on this patient.

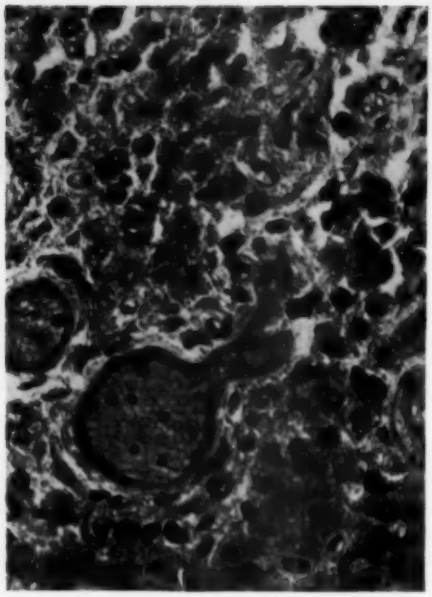

Fig. 1.—Testis. Nodule of interstitial cells; no seminiferous tubules are seen. Phloxine-methylene blue; × 320.

the external genitalia appeared normally developed.

The findings of principal interest were centered in the endocrine organs.

Testes.—The testes were normal in configuration but were markedly reduced in size, measuring 1.5×1×1 cm. On cut section, the parenchyma was reddish-brown in color and displayed an irregular nodularity. The seminiferous tubules could not be identified. The most prominent histologic finding was the presence of large nodular masses of interstitial cells (Figs. 1 and 2). These cells contained oval or round nuclei with finely divided chromatin material. No nuclear pleomorphism was noted, and there were no mitoses. The cytoplasm was eosinophilic and slightly granular in appearance and often had a pavement-like configuration. Many cells contained yellowish-brown pigment granules, some of which were Sudanophilic. Crystalloid bodies, although not diffusely distributed, were prominent in focal groups of cells. The nodules of interstitial cells contained a delicate vascular network and were surrounded by bands of loose fibrous connective tissue. Only occasional remnants of seminiferous tubules were encountered (Fig. 2). These consisted of dense hyaline masses containing a few fibroblastic nuclei. No Sertoli cells were identified. Sections of vas deferens showed almost complete obliteration of the lumen by dense hyalinized connective tissue. The prostate gland, normal in size, revealed a slight glandular and stromal hyperplasia.

Pituitary Gland.—The pituitary gland was normal in size and shape. The most prominent histologic alteration was a marked increase in the number of basophile cells in the pars anterior and the presence of numerous swollen chromophobes showing basophilic cytoplasmic stippling (Fig. 3). The pars intermedia was unremarkable. There was slight basophile infiltration of the pars posterior. Detailed cytologic studies are described below.

Thyroid Gland.—The thyroid gland weighed 14 gm. The cut surface was homogeneous, and there were no histologic abnormalities.

Fig. 2.—Testis. Two atrophic seminiferous tubules are shown. They consist of hyaline masses with a few fibroblastic nuclei. No Sertoli cells are seen. The surrounding tissue consists almost entirely of interstitial cells. Phloxine-methylene blue; × 240.

Fig. 3.—Adenohypophysis. A group of swollen chromophobes with basophilic cytoplasmic stippling occupies most of the field. A vacuolated basophile is shown in the lower left corner. Mallory's acid fuchsin, orange G, aniline blue; × 900.

Adrenal Glands.—The adrenal glands had a combined weight of 13.5 gm. Their histologic appearance was normal except for slight patchy lipid depletion. No abnormalities were encountered in the islets of Langerhans. The breasts and parathyroid glands were not examined histologically.

Autopsy findings relating to the patient's major illness consisted of massive cerebral hemorrhage involving the right frontal and the right parietal lobe, severe bilateral pulmonary emphysema, and chronic bronchitis. The heart was enlarged (525 gm.) with marked right ventricular hypertrophy and a 3 mm. high interventricular septal defect. There was evidence of terminal infection consisting of acute interstitial pneumonitis, interstitial myocarditis, and focal acute hepatic necrosis. Other findings included an acute ulceration of the duodenum, arteriolar nephrosclerosis, and a minimal degree of atherosclerosis of the major vessels.

STUDIES OF PITUITARY CYTOLOGY

The pituitary gland was fixed in 10% formalin and sectioned horizontally. Histologic sections were stained with hematoxylin and phloxine, Mallory's acid fuchsin, orange G, aniline blue, Pearse's trichrome-periodic acid-Schiff method,[6] and the modification of the periodic acid-Schiff technique described by Wilson and Ezrin.[7]

The differential cell count based on the aniline blue stain is shown in the Table. The striking increase in basophile cells is apparent. The percentages do not take into account the large number of swollen chromo-

Fig. 4.—Adenohypophysis. A large number of vesiculate cells is demonstrated by the Pearse trichrome-PAS technique; × 700.

Differential Cell Count of Anterior Pituitary Cells

	No. Cells Counted	Per Cent Acidophile	Per Cent Basophile	Per Cent Chromophobe
Patient D. M.	36,618	23.5	34.7	41.8
Mean values for adult males (17)	.....	37.0	11.0	52.0

phobes showing basophilic cytoplasmic stippling.

In a differential count of "mucoid" cells performed according to the method of Pearse, maximal cells comprised 28.8%, intermediate 54.3%, punctate 7.5%, vesiculate 4.6%, and disperse 4.8%. The maximal-intermediate ratio of 1 to 1.9 is indicative of increased hormone secretion. The large number of vesiculate cells is considered evidence of increased gonadotropin secretion.[8] A group of these cells is shown (Fig. 4).

Sections stained with the modified periodic acid-Schiff technique of Wilson and Ezrin failed to show a significant predominance of either PAS-red or PAS-purple cells, and the geographic distribution of the two cell types appeared to be similar.

No evidence of Crooke's hyaline change was encountered in sections stained with Mallory's aniline blue or Pearse's trichrome-periodic acid-Schiff method. Vacuolation of basophile cells, although occasionally marked in individual cells (Fig. 3), was not a generalized finding. The vacuoles did not take a positive stain in periodic acid preparations.

COMMENT

The observations of prime interest in our patient concern the testes and the adenohypophysis. The testes were markedly reduced in size and showed complete loss of active seminiferous tubules. Only a few hyalinized remnants of tubules were identified, and these were devoid of Sertoli cells. There was a distinct nodular hyperplasia of the interstitial cells of Leydig. These findings are similar to those described in the Klinefelter syndrome,[9] but additional clinical and laboratory evidence (pubertal onset, urinary gonadotropin and 17-ketosteroid excretion) would be necessary to make this diagnosis in our patient. We have no clue to the etiology of the testicular changes.

The adenohypophysis showed a marked increase in basophile cells (34.7%) and the presence of many swollen chromophobes with basophilic cytoplasmic stippling. Vacuolation of basophiles, although present, was not marked. These changes are compatible with those described following castration of experimental animals § and humans.[4] Application of the Pearse method showed a low maximal-intermediate ratio and an increased number of vesiculate cells, indicating an increased hormonal secretory activity of the "mucoid" series. This finding is in conformity with the known increased output of pituitary gonadotropin in the castrated animal.‖

An increase in pituitary basophiles is also characteristic of Cushing's syndrome. There was no clinical or morphologic evidence of this disorder in our patient. Although corticotropin administration can produce pituitary basophilism,[12] he received only 105 mg. of this hormone 13 months before death.

We have been unable to find reports of pituitary studies in patients with the Klinefelter syndrome. Wilbur and Burger,[13] however, reported a patient with seminiferous tubule atrophy and Leydig-cell hyperplasia. They found the pituitary basophiles increased in number but made no mention of basophile vacuolation. It is of interest that their patient, like ours, showed evidence of advanced pulmonary disease.

Recent studies in experimental animals strongly suggest the presence in the adenohypophysis of two types of basophiles having differing responses to altered hormonal balance. These cells have been designated as "gonadotrophs" and "thyrotrophs," [5] or as "beta" and "delta" cells.[2] Wilson and Ezrin, by modifying the periodic acid-Schiff technique, are able to distinguish "PAS-red" and "PAS-purple" cells in the human adenohypophysis.[7] We were able to identify these cells in our patient, but the two types appeared equally increased in number and were similar in geographic distribution.

The sites of estrogen and androgen production in the testis have long been a subject of controversy. It is now generally accepted that Leydig cells elaborate androgen. Most observers, headed by Howard and his co-workers,[14] name the Sertoli cells of the seminiferous tubules as the producers of estrogen. Maddock and Nelson,[15] however,

§ References 1 through 3.

‖ References 10 and 11.

studied the effects of chorionic gonadotropin administration and concluded that the Leydig cells elaborate both hormones.

The findings in our patient suggest that castration changes in the adenohypophysis are the result of the loss of Sertoli cells and that the presence of active Leydig cells does not alter this pituitary response. Clinical observations in patients with the Klinefelter syndrome support this concept. These patients show increased follicle-stimulating hormone (FSH) excretion in the urine. The administration of androgen, unless in very high dosage, does not alter normal or increased FSH excretion, but estrogen administration, even in small doses, results rapidly in decreased output of this gonadotropin.¶

Castration changes in the anterior pituitary would appear to result from a loss of estrogen secretion by the testis and to be a reflection of markedly increased gonadotropin production in this organ. The finding of extensive castration changes in a patient with loss of tubules, but normal or hyperplastic Leydig cells, points to the seminiferous tubules as the site of estrogen formation.

SUMMARY

Findings characteristic of castration are described in the adenohypophysis of a patient with seminiferous tubule atrophy and Leydig-cell hyperplasia. These changes are attributed to the loss of estrogen secretion resulting from the disappearance of Sertoli cells in this type of testicular atrophy.

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Experimental Aspiration Pneumonia

IV. Inflammatory and Reparative Changes Produced by Intratracheal Injections of Autologous Gastric Juice and Hydrochloric Acid

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Earlier reports on experimental aspiration pneumonia * have dealt with the effects of intratracheal injection of solid foods, milk, and sugar solutions on the lungs of rabbits and guinea pigs. The present report is concerned with the effects on rabbit lung of intratracheal injection of the animal's gastric juice and of several components of gastric juice.

Changes in human lungs following postoperative aspiration of gastric contents have been described by Apfelbach and Christianson [4] and by Irons and Apfelbach.[5] Fetterman and Moran [6] described the changes in human lungs after aspiration of solid food during the act of swallowing or from aspiration of gastric contents while vomiting. Teabeaut [7] described the pneumonia produced by intratracheal injection into rabbits of various test meals, after they had been eaten by a human volunteer and then withdrawn through a stomach tube. Teabeaut also injected hydrochloric acid into rabbit lungs and described the changes produced through the first 21 days after injection. He reported that the production of pneumonia depended on the pH and volume of the injected material and that solutions of pH value above 2.4 did not cause pneumonia, although foreign-body reactions occurred around injected food or milk.

No attempt was made in the present study to determine the effects of solutions of different pH values, but studies were made of lung changes at different time intervals after intratracheal injections of the animal's gastric contents, hydrochloric acid at pH values of normal rabbit gastric juice, and pepsin and steapsin.

METHODS

Intratracheal injections of gastric contents, hydrochloric acid, pepsin, and steapsin solutions were given to 49 rabbits under light ether or pentobarbital (Nembutal) anesthesia. The gastric contents were obtained in some instances by operative incision of the abdomen and removal of the stomach contents by a needle and in other instances by insertion of a stomach tube. Half of the animals had been without food for two days prior to obtaining the gastric contents; the others had been on their normal diets. Portions of the gastric contents of some of the animals were injected directly into the trachea; several specimens were filtered before injection, and other specimens were centrifuged and the clear supernatant fluid injected. The pH of the rabbit gastric juice as determined by the Beckman pH meter ranged from 1.0 to 2.3, with most specimens falling in the 1.1 to 1.3 range. The material injected and the fate of the animals are shown in the Table. The animals were killed at varying time intervals after injection by introducing air into the marginal ear veins, unless death occurred before the scheduled time. Autopsies were performed immediately after death, except in two instances in which death occurred during the night and the autopsies were done in the morning.

At least six blocks of tissue from different portions of both lungs were fixed in 4% formaldehyde solution. Paraffin sections of all blocks were stained by hematoxylin and eosin methods. Masson's trichrome and Verhoeff's elastic tissue stains were used in selected cases. Portions of several lungs were cut by frozen-section technique and were stained with Sudan IV.

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* References 1 through 3.

Control animals were available from previous experiments. These included nine rabbits given intratracheal injections of 5 cc. of sterile distilled water and six rabbits given intratracheal injections of 5 cc. of sterile 0.9% sodium chloride solution. Lungs of more than 250 rabbits and guinea pigs, injected intratracheally with solid foods, milk, or sugar solutions in other experiments † were also available for comparison. Lungs of numerous normal rabbits killed by intravenous air injections were also studied.

RESULTS

Fifteen of the 49 rabbits died as a result of their injections. Nine of the 19 animals injected with gastric juice died at intervals from 6 minutes to 25 days after injection. Five of the 17 rabbits given injections of hydrochloric acid (pH 1.1 or 1.4) died in 20 hours to 12 weeks. One of four animals given 5 cc. of steapsin in hydrochloric acid (pH 1.4) died four minutes after injection. The 34 rabbits which did not succumb to the injections were killed at intervals varying from 24 hours to 6 months.

Data on Material Injected and Time of Death

Material and Amount	No.	Died	Killed
Gastric juice, 2 cc. or less	13	6 min., 18 hr., 24 hr., 21 da., 25 da.	2 da., 2 wk., 4 wk., 6 wk. (3), 11 wk., 20 wk.
Gastric juice, 5 cc	4	12 min., 4 da.	6 wk., 10 wk.
Gastric juice, 7 cc., 10 cc	2	20 min., 90 min.	
HCl (pH 1.4), 1 to 5 cc	4		24 hr., 48 hr., 1 wk., 2 wk.
HCl (pH 1.4), 5 cc., 1 cc. (injected once weekly)	2	23 da., 12 wk.	
HCl (pH 1.1), 2 cc	8		3 wk., 4 wk. (2), 6 wk., 8 wk., 12 wk., 6 mo. (2)
HCl (pH 1.1), 5 cc	3	20 hr. (2), 2 da.	
Pepsin (0.4% in H_2O, pH 4.3), 5 cc	3		2 da., 1 wk., 2 wk.
Pepsin (0.4% in HCl, pH 1.4), 5 cc	3		2 da., 1 wk., 2 wk.
Steapsin (0.4% in H_2O, pH 5.8), 5 cc	3		2 da., 1 wk., 2 wk.
Steapsin (0.4% in HCl, pH 1.4), 5 cc	4	4 min.	2 da., 1 wk., 2 wk.

The pulmonary changes could be divided into three principal groups: (1) acute pulmonary edema, (2) acute hemorrhagic pneumonia, (3) chronic inflammatory and reparative lesions.

1. *Acute Pulmonary Edema.*—Pulmonary edema was the cause of death in the five animals dying in 4 to 90 minutes after injection. Four of these deaths occurred in animals injected with gastric juice, and one, in an animal injected with steapsin in hydrochloric acid. The animals developed dyspnea, rapid respirations, and convulsive seizures, and frothy pink fluid appeared at the nares. The gross findings were similar to those in earlier experiments on the production of acute pulmonary edema by intratracheal injection of milk, feeding mixtures, and carbohydrates,[8] except that frank hemorrhage was much more conspicuous in the animals injected with gastric juice (Fig. 1). The hemorrhages were especially prominent along the course of the main bronchi. The trachea and bronchi of all five animals were filled with frothy pink or red fluid, and the same fluid dripped from the cut surfaces of the lung parenchyma. The weight of the lungs and trachea varied from 11 to 33 gm., as compared with normal weights in our animals of 5 to 10 gm. As in the earlier experiments on acute pulmonary edema, the lighter lungs were from animals dying quickly, and the heavier lungs were from animals surviving for longer periods.

Microscopically, edema and hemorrhage were found. The edema fluid was often inconspicuous and not nearly so easily detected as the pulmonary edema of long duration

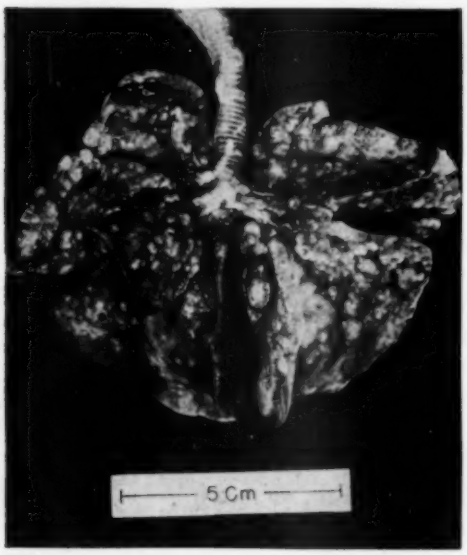
† References 1, 2, 3, and 8.

Fig. 1.—Acute pulmonary edema and hemorrhage in rabbit which died 12 minutes after injection of 5 cc. of the arimal's gastric juice.

associated with congestive heart failure in human lungs.

2. *Acute Hemorrhagic Pneumonia.*—Six rabbits died of acute hemorrhagic pneumonia in 18 hours to 4 days after injection. Changes in rabbits which died of pneumonia and in those killed in 18 hours to 4 days differed only in degree. Lungs with hemorrhagic pneumonia weighing 25 to 42 gm. were observed. The distribution and extent of the pneumonia varied considerably, and there was no predilection for one side or the other. Empyema was found in one animal, which died in four days. Areas of collapse and emphysema were seen in all. The severest pneumonias were seen in animals injected with gastric contents or hydrochloric acid.

Fig. 2.—Multiple abscesses and granulomas in rabbit lungs 12 weeks after once weekly injections of 1 cc. of HCl (pH 1.4).



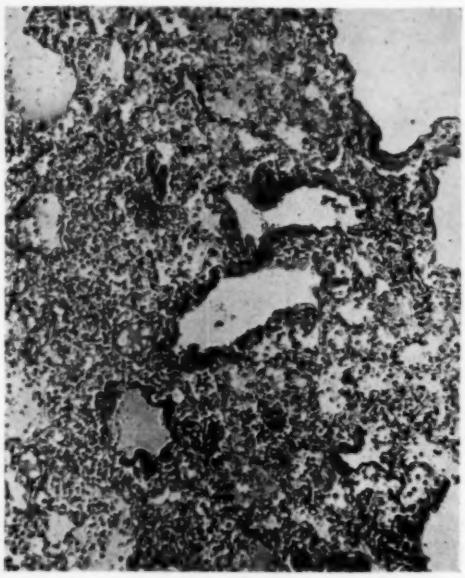


Fig. 3.—Hyaline membranes in acute pneumonia two days after injection of 5 cc. of HCl (pH 1.1). Hematoxylin and eosin; reduced about ⅓ from mag. × 140.

Only minimal changes were observed in animals receiving pepsin in water (pH 4.3). In animals receiving pepsin in hydrochloric acid, changes similar to those in animals receiving hydrochloric acid alone were found. This finding corroborated Teabeaut's impression [7] that the pepsin of the gastric juice is not an important factor in the production of aspiration pneumonia.

Microscopic findings in this group of animals included an acute hemorrhagic pneu-

monia with edema fluid, red blood cells, and granulocytes. In several instances well-formed hyaline membranes were a prominent feature of the reaction (Fig. 3). Bronchial and bronchiolar ulceration was found in most animals. The ulceration first involved the epithelial lining and then extended into the submucosa and muscularis. Early interstitial and mononuclear pneumonia was found in animals which died or were killed in two to four days. In one animal, which died in four days, an early fibroblastic reaction was found in several areas. Vascular changes in these animals included venous and arterial thrombosis, acute vasculitis, and necrosis of many small vessels. The thrombi were often acellular and hyaline.

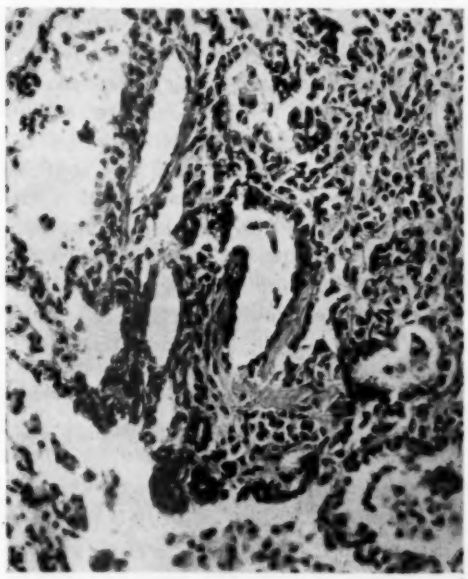
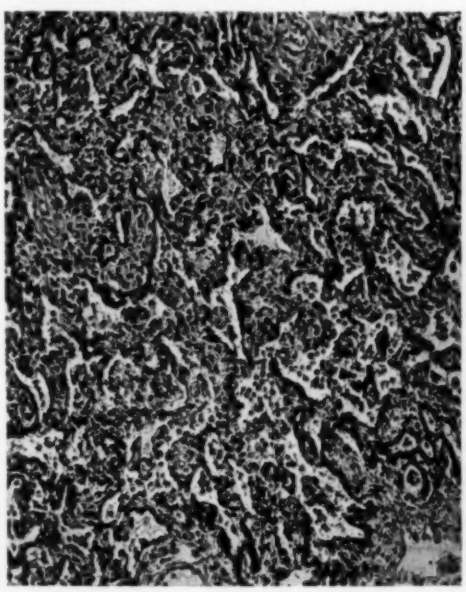


Fig. 4.—Central space lined partly by regenerating epithelium and partly by fibroblasts. Early interstitial fibrosis is present. One week after injection of 5 cc. of 0.4% steapsin in water (pH 5.85). Hematoxylin and eosin; reduced about ⅓ from mag. × 280.

3. *Chronic Inflammatory and Reparative Lesions.*—Four rabbits died 3 to 12 weeks after injection. Changes in animals which died or were killed in one week to six months varied considerably. The animals which died showed extensive lung damage with pneumonia and abscess formation (Fig. 2). There

Fig. 5.—Fibrosis of bronchiolar muscularis and regeneration of epithelium in bronchiole and in satellite fashion around it, in areas corresponding to location of smaller bronchioles. Animal killed 10 weeks after injection of 5 cc. of its gastric juice. Hematoxylin and eosin; reduced about ⅓ from mag. × 140.

Fig. 6.—Interstitial fibrosis and bronchiolar regeneration in rabbit killed 12 weeks after injection of 2 cc. of HCl (pH 1.1). Hematoxylin and eosin; reduced about ⅓ from mag. × 140.



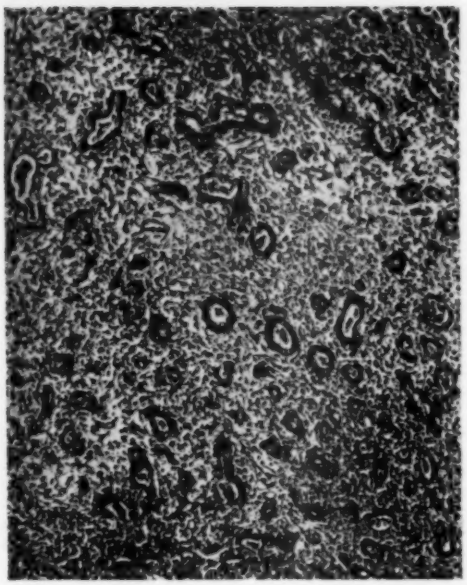


Fig. 7.—Area of atypical bronchiolar regeneration and fibrosis, with superficial resemblance to secondary carcinoma. Animal killed 10 weeks after injection of 5 cc. of its gastric juice (pH 1.3). Hematoxylin and eosin; reduced about ⅓ from mag. × 140.

was green or greenish-blue discoloration of the pneumonic portions of several lungs. The lungs of many of the animals which had received only injections of hydrochloric acid contained large and small particles of food. These animals, prior to death, had developed difficulty in swallowing. Several had been observed to choke even while drinking water. Food particles had not been found in lung sections of numerous control animals previously studied.

The pneumonia observed was usually interstitial and mononuclear, and numerous granulomatous areas were scattered through many sections. These granulomas were often interspersed with large and small abscesses. The granulomatous areas and the abscesses were often partially or completely surrounded by a zone of fibroblasts or by dense fibrous tissue. The granulomas were often found around pink-staining material resembling necrotic fibrous tissue and smooth muscle, apparently from the walls of the bronchi and blood vessels. Well-formed hyaline membranes were found in several animals with pneumonia and abscess formation. Empyema was found in several animals. In occasional areas, collections of large, finely vacuolated macrophages resembled those seen in the human lesion ordinarily designated as "endogenous lipoid pneumonia."

Bronchial changes in the animals surviving 1 to 13 weeks included ulceration and regeneration. In animals with severe pneumonias and abscesses many bronchi were completely necrotic, others were only faintly outlined, and others were lined only by a single layer of reserve cells. When regeneration occurred, frequent "buds" of epithelium extended out from the walls, especially of the larger bronchioles, and in some instances these broke off to form masses in the lumen resembling "foreign-body" giant cells. Bronchiolar regeneration in many instances was atypical, with irregular gland formation and occasional mitotic figures. The bronchiolar epithelium often extended irregularly into the damaged portions of the lung, and the combination of the bronchiolar regeneration and

Fig. 8.—Another area of atypical regeneration of bronchiolar epithelium resembling tumor. Animal killed 12 weeks after injection of 2 cc. of HCl (pH 1.1). Hematoxylin and eosin; reduced about ⅓ from mag. × 140.

the interstitial fibrosis produced localized areas resembling the reaction in human lungs described by Hamman and Rich [9] as "acute diffuse interstitial fibrosis" (Fig. 6). In some areas the regenerating epithelium, surrounded by dense fibrous tissue, superficially resembled neoplasm (Figs. 7 and 8).

The extension of the bronchiolar epithelial cells into damaged areas gave a superficial appearance of cuboidal epithelium lining alveolar spaces (Fig. 4). Careful studies of such areas led to the conclusion that these were not functioning alveolar spaces but "pseudoalveoli" formed by young fibrous tissue and capillaries. These spaces were also lined in many instances by macrophages or fibroblasts. The spaces lined by definite epithelium were usually in satellite clusters around large bronchioles, the appearance and location suggesting that the cells were derived from regeneration of terminal bronchioles (Fig. 5). Spaces lined by macrophages or fibroblasts were usually farthest from the larger bronchioles. In no instance was there evidence to support the concept of a true epithelial alveolar lining.

The larger bronchioles and bronchi often showed lymphoid follicles or fibrous tissue, not covered by epithelium, extending into the lumens. In many instances the submucosa and muscularis were completely replaced by fibrous tissue containing inflammatory cells. Large lymphoid collections were also seen in the wall, and these occasionally surrounded small glands or bits of partially necrotic smooth muscle from the muscularis.

The vascular changes in these animals included spotty venous and arterial thrombosis, necrosis of vessels, acute or chronic vasculitis, and endarteritis. These changes were always found in areas of inflammation or repair. Numerous granulocytes were found in the adventitia and in the intima of involved vessels, and occasional small collections of granulocytes were found in the media in relatively early stages of the vasculitis (Fig. 9). In late stages the intima was thickened and the lumen was narrowed. The internal elastic lamella was frequently disrupted. Numerous small collections of lymphocytes, monocytes, and red blood cells were found in small clear spaces in the wall of the vessels, usually between the media and the intima. Perivascular inflammation and fibrosis were common.

Fig. 9.—Acute vasculitis in rabbit, six weeks after injection of 2 cc. of animal's gastric juice (pH 1.0). Hematoxylin and eosin; reduced about ⅓ from mag. × 140.

Areas of fibrosis, emphysema, and collapse were numerous in these animals. Approximately half of the animals showed severe changes, and half showed only minimal changes, including areas of mononuclear pneumonia, small granulomatous foci, and occasional small scars.

Severity of Reactions to Different Materials

The severity of the reactions varied somewhat in all groups, but animals receiving gastric juice or hydrochloric acid showed the severest changes. Nine of the 19 animals injected with gastric juice died in 6 minutes to 25 days. One animal, killed in 2 days, had an early but severe pneumonia, and one animal, killed in 11 weeks, had an extensive pneumonia with empyema. The remaining eight animals injected with gastric juice had only mild pneumonic reactions or focal granulomas with slight fibrosis and focal vascu-

litis. Five of the 17 animals injected with hydrochloric acid died in 20 hours to 12 weeks; 3 animals had severe pneumonic reactions with abscess formation or empyema, and the remaining animals had mild pneumonia or minimal granulomatous reactions with fibrosis and vasculitis. The three animals injected with pepsin in hydrochloric acid (pH 1.4) had severe changes comparable to those seen in animals receiving comparable amounts of hydrochloric acid alone. Two of the animals injected with pepsin in water had minimal pneumonic reactions, and the lungs of the third animal were normal. One of four animals injected with steapsin in hydrochloric acid (pH 1.4) died in 4 minutes of acute pulmonary edema; one had a severe pneumonia, and the other two had only minimal changes. Two of three animals injected with steapsin in water (pH 5.8) developed severe pneumonia; the third had only a minimal focal acute reaction.

COMMENT

The early changes produced in rabbit lungs by injection of gastric juice and hydrochloric acid in this experiment are similar to those described by Teabeaut.[7] Certain changes not previously described were found in animals observed over longer periods of time. The interstitial fibrosis, the atypical bronchiolar regeneration, and the areas of scarring containing atypical epithelium were suggestive of many reparative lesions of the human lung. In many areas the combination of atypical bronchiolar regeneration and fibrosis resulted in an appearance superficially resembling a neoplasm. Such areas might be mistaken for "pulmonary adenomatosis" or "secondary carcinoma." The presence of hyaline membranes in early and late pneumonic reactions points out again that this finding cannot be used to establish the viral or other specific etiology of a pneumonic reaction.

In rabbits dying of acute pulmonary edema, the gross appearance of frothy pink or red fluid in the bronchi was quite characteristic. The microscopic picture, on the other hand, often showed little evidence of edema and no specific changes to suggest the cause of the edema. The explanation of the minimal microscopic evidence of edema is probably related to the protein content of the edema fluid. In a previous experiment,[8] it was demonstrated that the protein content of the edema fluid and the weight of the lungs were greater in animals which survived for longer periods of time. Lungs of animals which died within a few minutes were relatively light, and the protein content of the edema fluid was less. In general, the edema fluid was easier to demonstrate microscopically when the protein content was high. When acute pulmonary edema is found in human subjects, the possibility of aspirated gastric contents should be considered as a possible cause of the pulmonary edema.

SUMMARY

The effects of intratracheal injection of autologous gastric juice, hydrochloric acid, pepsin, and steapsin in rabbits have been studied. The changes produced include acute pulmonary edema and acute hemorrhagic pneumonia, both often fatal, and a variety of inflammatory and reparative lesions occurring after the first week, some of which also caused death. Five of the 49 animals in the series died of acute pulmonary edema in 4 to 90 minutes. Acute hemorrhagic pneumonia caused the death of six animals in two to four days. Death in four other animals occurred in 3 to 12 weeks.

The changes occurring in animals dying or killed in 1 to 26 weeks included abscess formation, mononuclear and interstitial pneumonia, granulomatous reactions, interstitial thickening and fibrosis, atypical bronchiolar regeneration, and vasculitis. Combinations of atypical bronchiolar regeneration and interstitial or diffuse fibrosis often produced localized areas resembling "diffuse interstitial fibrosis," "pulmonary adenomatosis," or "secondary carcinoma." Hyaline membranes were found in several pneumonic reactions occurring one day to several weeks after injection. Aspiration of food occurred in many animals receiving injections of hydrochloric acid, suggesting that a primary

respiratory disease may predispose to aspiration pneumonia even in patients not in a recumbent position.

The findings reported suggest that a variety of unexplained inflammatory, reparative, and fibrotic lesions of the human lung may be caused by aspiration of foreign material, especially gastric contents. Certain unexplained instances of acute or subacute pulmonary edema, especially in infants, may also be caused by aspirated gastric material.

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The Hypervitaminosis-A Syndrome in Adrenalectomized Rats

Skeletal Changes in Weanling Rats With and Without Cortisone

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The purpose of this study was to ascertain if the adrenal glands are intermediators in the production of the skeletal response to excessive administration of vitamin A. The manner of action of hypervitaminosis A on the skeleton of weanling rats, which consists of a distinctive pattern of acceleration of certain growth processes, has been noted, in a previous study,[1] to have an integration resembling that of a hormonal mechanism. That study, to which this is a sequel, showed that certain details of the histological pattern were slightly altered in the hypophysectomized animals. It seemed of interest to discover whether hormonal mediation, other than pituitary, of the skeletal changes due to hypervitaminosis A might occur. The purpose of the present study was to ascertain if adrenalectomy or cortisone influenced the skeletal changes elicited by hypervitaminosis A in the weanling rat.

EXPERIMENTAL DATA

The experiment utilized 49 male rats of Sprague-Dawley strain, which were 32 days old and weighed 66 to 87 gm. Thirty of these had been adrenalectomized * at 28 days of age. Adrenalectomized and intact animals were grouped as follows (Table 1): (*a*) those receiving vitamin A; (*b*) those receiving vitamin A plus 1 mg. of cortisone per kilogram of body weight, and (*c*) those receiving vitamin A plus 50 mg. of cortisone per kilogram of body weight. Additional groups were run, corresponding to the above in all respects except that they received no vitamin A.

In order to maintain the weight of intact animals in any one group approximately equal to those of the corresponding adrenalectomized group, an attempt was made to keep the average food intake of the two groups equal. However, since the operated rats, given vitamin A, responded more promptly than intact rats by diminishing their food intake and losing weight, the scheme could not be followed consistently without imposing starvation on the control group, and, therefore, after a two-day trial it had to be abandoned. Some limitation of food intake was still maintained in the intact animals so as to keep comparable the average weights of corresponding groups.

All 49 rats were kept in individual cages with wire bottoms in a well-ventilated room maintained at 80 F. The adrenalectomized rats that received no cortisone were given a 1% solution of sodium chloride for drinking water, while those that received cortisone were given tap water. Intact rats that received cortisone were given supplementary potassium chloride at a level of 20 mg. per milligram of cortisone.

The diet was the same as that employed in a previous study,[1] except that brewer's yeast was substituted for synthetic B vitamins at a level of 50 mg. per kilogram of diet. Thirty thousand units of vitamin A and 5000 units of vitamin D were incorporated in the peanut oil of each kilogram of diet.

Cortisone † was used at two dosage levels; 1 mg. and 50 mg. per kilogram of body weight was given

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† Dr. Wolbach died March 19, 1954.

* All rats, intact and adrenalectomized, were obtained from Hormone Assay Laboratories, Inc., Chicago.

† Cortone ® acetate (11-dehydro-17-hydroxycorticosterone-21-acetate) in saline suspension 25 mg./ml. obtained from Merck & Company, Inc.

TABLE 1.—*Effects of Cortisone and Hypervitaminosis A on Survival of Adrenalectomized and Intact Rats*

Category*	No. Rats	Cortisone Dose, Mg./Kg./Day	Average Daily Weight Change, Gm.	Seven-Day Survival, Per Cent	Average Survival Time, Days		Total Dose Vitamin A, I.U. × 1000	
					Died	Killed	Died	Killed
Ad + vit. A	12	..	−1.2	25	4.6 (9)†	6.7 (3)	160	247
N + vit. A	3	..	+1.6	100	.....	6.7 (3)	...	212
Ad + vit. A	2	1	−3.3	0	4.0 (2)	.....	184	...
N + vit. A	3	1	+0.8	100	.....	13 (3)	...	510
Ad + vit. A	3	50	−2.2	100	10 (2)	6 (1)	327	280
N + vit. A	3	50	−0.6	100	10 (2)	6 (1)	324	238
Ad	3	1	−0.3	67	5 (1)	20 (2)	...	...
N	3	1	+2.2	100	.....	17 (3)	...	...
Ad	3	50	+1.0	100	.....	19 (3)	...	...
N	3	50	+3.2	100	.....	19 (3)	...	...
Ad	4	..	+1.9	75	5 (1)	8 (3)	...	...
N	3	..	+5.4	100	.....	18.7 (3)	...	...

* Ad refers to adrenalectomized animals; N, intact animals.
† Figures in parentheses indicate number of animals in group.

orally. The lower dosage was chosen to represent adequate substitution, while the higher one represented a significant level of overdosage.[2]

Vitamin A‡ was administered in the form of the palmitate at a level of 1000 units per gram of body weight for the first day, but the toxic effects of this dose were so great in adrenalectomized rats that it was halved for subsequent doses to allow sufficient time for skeletal lesions to develop. The same schedule was followed for the control rats.

In order to study the effects of hypervitaminosis A on adrenalectomized rats of longer standing, two 42-day-old rats (#47 and #48, Table 2), which had been adrenalectomized at 28 days of age, were used. These animals received 1000 units of vitamin A daily for the period of their survival, i. e., three days.

A complete postmortem examination was done on each animal, with emphasis grossly on ascertaining if adrenalectomy had been complete. Those rats that were killed were anesthetized with ether and bled by cardiac puncture, and the blood samples so obtained were analyzed for serum mucoprotein (tyrosine) by the method of Winzler. The histological techniques used were the same as in a previous study, and, whether death was spontaneous or by killing, histological preparations were made from all major viscera and the skeleton of each animal.

RESULTS

The toxic effects of hypervitaminosis A were severer and occurred more promptly in the adrenalectomized than in the intact rats. While intact animals continued to gain weight, although at a subnormal rate, the adrenalectomized animals, immediately after the first dose of vitamin A was administered, became anorectic and either failed to gain or began to lose weight. The number of animals in each category was too small to yield data capable of statistical analysis with regard either to survival time or to weight curves; therefore, only gross changes were deemed significant. An indication of the differences in survival time (Table 1) charted arbitrarily for the seventh day of the experiment showed marked lowering in survival time of adrenalectomized animals that received vitamin A. Cortisone at the 1-mg.-per-kilogram level did not prolong survival, while at the 50-mg.-per-kilogram level it did. The earliest fatalities in each of these three categories were at 2, 4, and 10 days, respectively, while the average survival of the animals that died were, respectively, 5, 4, and 10 days. The weight curves also indicated decreased tolerance for vitamin A by adrenalectomized animals and are exemplified in Table 1 arbitrarily at the seventh day of the experiment, the averages including terminal weights of those animals that succumbed prior to that day. It is evident that cortisone at either of the dosage levels used did not mitigate the weight loss, and, if anything, made the animals lose more weight. This was true particularly of the intact animals, whereas in the adrenalectomized animals the data pointed toward a similar

‡ Supplied by Hoffmann-LaRoche, Inc., as a synthetic palmitate ester containing 1,000,000 I.U./gm., which was diluted one-fifth in peanut oil for greater accuracy in administration.

conclusion but were obscured by the short survival time of some of the animals.

The total amounts of vitamin A received by each group of rats are recorded in the last two columns of Table 1. Since these amounts were dependent on the survival times of the animals and on their initial and subsequent weights (daily dosage after the initial dose of 1000 units per gram being 500 units per gram of body weight), they serve to show the low levels of dosage that produced the specific histological effects, and, in addition, the general toxicity in adrenalectomized groups compared with corresponding categories of intact animals. The last column of Table 1, included for completeness, presents the total dosages in the killed animals. Killing was carried out at appropriate stages of the experiment to provide proper material for the study of histological sequences. Thus, the data do not specifically indicate variation in vitamin-A effect elicited by varying the level

TABLE 2.—*Data on Individual Rats, Intact or Adrenalectomized, with Respect to Weight, Fractures and Survival*

Rat, No.	Category*	Terminal Age	Total Dose Vitamin A, I.U. × 1000	Cortisone Dosage, Mg./Kg.	Initial Weight, Gm.	Weight Change, Gm.	Fractures	Survival, Days
8A.....	Ad	33 D†	68	0	68	0	0	1
10A.....	Ad	33 D	70	0	70	0	0	2
11A.....	Ad	36 Km‡	119	0	81	−10	+	4
7A.....	Ad	36 D	120	0	84	−14	0	4
6A.....	Ad	37 D	150	0	76	− 6	+	5
12A.....	Ad	37 Km	173	0	71	− 5	+	5
3A.....	Ad	38 K§	203	0	87	−14	+	6
4A.....	Ad	39 Km	252	0	84	− 2	+	7
1A.....	Ad	39 K	239	0	85	+ 3	+	7
2A.....	Ad	39 Km	229	0	83	−12	+	7
5A.....	Ad	39 K	260	0	82	− 2	+?	7
9A.....	Ad	39 D	250	0	85	− 5	+	7
15A.....	N*	38 K	172	0	66	+13	0	6
13A.....	N	39 K	229	0	75	+11	0	7
14A.....	N	39 K	235	0	77	+ 9	0	7
16A.....	Ad	36 D	196	1	82	− 0	0	4
17A.....	Ad	36 D	172	1	74	−17	0	4
18A.....	Ad	42 D	422	1	78	+12	+	10
20A.....	N	42 K	376	1	66	+ 7	+	10
21A.....	N	42 K	348	1	77	+ 9	+	10
19A.....	N	51 K	866	1	80	+33	+	19
24A.....	Ad	38 K	280	50	84	−14	+	6
22A.....	Ad	42 D	315	50	79	+23	+	10
23A.....	Ad	42 D	339	50	85	−24	+	10
25A.....	N	38 K	238	50	70	− 2	+?	6
26A.....	N	42 K	308	50	71	−11	+	10
27A.....	N	42 K	340	50	76	− 6	+	10
41A.....	Ad	37 Km	...	0	69	− 1	0	5
40A.....	Ad	39 K	...	0	75	+20	0	7
42A.....	Ad	39 K	...	0	80	+11	0	7
46A.....	Ad	42 D	...	0	69	+11	0	10
47A.....	Ad	45 D	328	0	107	− 8	0	3
48A.....	Ad	45 D	318	0	105	−10	0	3

* Ad refers to adrenalectomized animals; N, intact.
† Died.
‡ Killed in moribund condition.
§ Killed.

of dosage, although its existence is not improbable.

The histological changes that occurred in the adrenalectomized rats were not very different from those occurring in intact rats of corresponding category, except that the changes were seen at much lower levels of dosage and after shorter periods of hypervitaminosis A. In intact rats given vitamin A shortly after weaning, fractures rarely occur before the fifth day, when a dosage level of 1000 units per gram of body weight is used. Although the rats in this experiment were slightly (four days) older at the starting point of administration of vitamin A to allow a period of recovery from adrenalectomy, a few fractures occurred by the fifth day with use of a dose about half the usual, i. e., 500 units per gram (Table 2), and fractures were uniformly evident by the seventh day, while in intact animals on the same dosage schedule no fractures occurred until after the seventh day. Cortisone did not seem to influence the number or time of appearance of fractures in either the intact or the adrenalectomized categories.

The histological features of the skeletons of the adrenalectomized rats that received no vitamin A, as shown by sagittal-plane sections of the knee joint, were entirely within the limits of variation of the normal. In the sections from intact as well as adrenalectomized rats that received cortisone but no vitamin A, definite but nonspecific effects could be seen, which have been described as occurring in inanition as well as in other conditions. These changes were present to a small degree in those animals that received 1 mg. of cortisone per kilogram of body weight daily but to a much more marked degree in those receiving 50 mg. These changes have previously been summarized [1] as

> pronounced retardation of the multiplication, growth, and maturation of the epiphyseal cartilage cells with consequent diminution of the thickness of the cartilage plate owing to the decrease in size and number of the cells and a very great reduction in the numbers of capillaries penetrating from the diaphyseal side. . . . Remodeling sequences in our preparations were reduced in activity as estimated by persistence of bone and reduction in numbers of osteoclasts in regions where, in normal growth, resorption of bone is most active. In general, the effects of hypophysectomy upon the epiphyseal cartilage of young rats are similar to those of aging, severe inanition, and vitamin-A deficiency—the differences being mainly those of tempo.

In the present series, the changes were not so severe as those described by Becks and other investigators § for hypophysectomized rats. The degree of change in general was correlated not only with the dose level of cortisone but with the degree of inanition and with the duration of the experimental interval for each animal, and so, considering only these three factors, no single one could be shown to outweigh the others.

The histological changes that result from hypervitaminosis A have been described [4] by one of us (S. B. W.) and may be summarized as follows: The cells of the epiphyseal cartilage show accelerated growth and maturation; there is thinning of the cartilage layer due to excessive invasion by cells and capillaries of the marrow. Other accelerated evidences of remodeling sequences are seen in the spongiosa, where tongues of calcified cartilage matrix reach farther into the marrow than in the normal, and in the diaphysis, where much of the compact bone may be replaced by endosteal or periosteal bone of less mature configuration. Weakening of the diaphysis results from this rapid remodeling sequence and is responsible for fractures, which may occur in any portion of the diaphysis.

No significant differences could be observed in the changes in epiphyseal cartilage when adrenalectomized animals given vitamin A were compared with intact animals of corresponding category. Cortisone, at either of the dosage levels used, did not seem to have a significant influence on these changes. In the remodeling sequences in the spongiosa and in the diaphysis, the adrenalectomized animals did not elaborate as much endosteal or periosteal bone as did the intact animals of corresponding category, and this phenomenon was also evident in the callus of fractures, which was more abundant in the intact animals. A general inverse correlation existed

§ References 2 and 3.

between the degree of inanition of the animals and the amounts of endosteal bone or callus produced, and no additional influence of cortisone could be seen other than that attributable to the superimposed inanition. It must be pointed out that study of repair of fractures as influenced by cortisone in this series was limited by the haphazard distribution of the fractures with respect to location and to the period of repair. Therefore, while thoroughgoing differences in repair ascribable to cortisone could not be made out, effects of lower degree may have existed that we were unable to notice because of the limitations of the material. Concerning changes occurring in other tissues or organs than those mentioned above, no findings that have not been recorded previously either for hypervitaminosis A, for adrenalectomy, or for cortisone effect were elicited.

The determinations of serum mucoprotein which were made on those animals that were killed, although few in number, seem worthy of record, inasmuch as they provide one more instance || of elevated values where dissolution of skeletal tissues was a fundamental component of the pathological process. The average values, expressed as milligram per 100 ml. of serum, followed in parentheses by the number of rats on which determinations were done are as follows: (*a*) for intact rats that received only vitamin A, 5.7 [3]; (*b*) for intact rats that received vitamin A plus 1 mg. of cortisone per kilogram of body weight, 9.0 [2]; (*c*) for intact rats that received 50 mg. of cortisone per kilogram of body weight, 7.7.[3] A single determination on an adrenalectomized rat yielded a value of 9.4, while values on two adrenalectomized rats that received vitamin A alone and vitamin A plus 50 mg. of cortisone per kilogram were 14.2 and 10.6, respectively.

COMMENT

Although it was suggested in previous experiments with hypophysectomized rats that hypervitaminosis A effects could not be mediated through the adrenal glands, since the latter showed atrophic changes in those animals, it was thought that adrenalectomy would provide a more direct demonstration of that hypothesis. Thus it may now be stated that the skeletal changes in hypervitaminosis A are not mediated either through the adrenal glands or through the pituitary. Allowing for the decreased ability of adrenalectomized rats to form new bone, we find no differences in their responses to excessive vitamin A as compared with those in normal rats, and, as in the hypophysectomized series, the earlier incidence of fractures may be explained by this decrease in new-born formation. We must point out that comparison of the data on hypophysectomized versus adrenalectomized rats subjected to hypervitaminosis A, once the difference in dosage level is taken into account, shows very similar responses with respect to survival time, incidence of fractures, and inanition (toxic) effect. The histological phenomenon of premature sealing off of the epiphyseal cartilage was attributable to hypophysectomy and did not occur in the adrenalectomized series, although comparable diminution in appositional bone formation occurred in both ablation experiments. It seems probable that this lowered appositional proliferation in response to hypervitaminosis A in both series as compared with intact rats is due either to the loss of adrenal function common to these animals, or to the inanition seen in them, or to a combination of the two factors. Inanition of comparable degree for the same experimental interval could not be reproduced in intact animals except when an additional toxic agent, in this case cortisone at the 50-mg.-per-kilogram level, was given with the vitamin A. However, if cortisone produced any change in the amount of appositional bone formed, one would expect a diminution rather than an augmentation. Therefore, the occurrence of more appositional bone in intact animals given cortisone plus vitamin A as compared with that in adrenalectomized animals given only vitamin A, both series showing comparable weight curves, indicates that this phase of the response to hypervitaminosis A is influenced to some extent by adrenal functions other than cortisone production, but is not

|| References 5 and 6.

mediated exclusively through the adrenal glands, since, in their absence, considerable appositional bone still was evident.

The histology of the skeletal sections of the control animals in the category of adrenalectomy merits a passing comment. No widening of the epiphyseal cartilage occurred as described by Wyman and Tum-Suden,[7] but such was not to be expected after the short periods of observation which our experiments provided, and our results were similar to their short-term results in all respects. The histology of the epiphyseal cartilages in the categories of intact or adrenalectomized control rats that received 50 mg. of cortisone per kilogram of body weight did not correspond to that elicited by Follis.[8] He described a persistence of a lacework of calcified cartilage trabeculae reaching from the epiphyseal cartilage into the metaphysis, which did not occur in any animal of our experiment that received cortisone. We must point out, however, that our experimental conditions differed from his, in that our normal rats that received cortisone also received 20 mg. of potassium chloride per milligram of cortisone, whereas the adrenalectomized group did not—both categories received a diet markedly different from that used by Follis,[8] and these factors may explain the discrepancy.

When the question of the manner of action of vitamin A on the skeleton is considered in the light of these experiments, it is of interest to note that the pattern of reaction may be altered to the extent that the catabolic component (consumption of epiphyseal cartilage) may proceed at the usual rate, while the anabolic (formation of appositional bone) is inhibited. We still believe that the essential pattern is one of acceleration of normal sequences but that, in the adrenalectomized (or hypophysectomized) animals, the processes of tissue synthesis, whether in growth or repair, are inhibited. Whatever the mechanism by which this pattern of response is fashioned, it is clear that the medication of the adrenal glands is not necessary for it, and, although we are no wiser concerning the site of action on the skeleton of vitamin A, a metabolic pathway is indicated that may well be local in character. Other experiments, now in progress, which involve ablation of other endocrine glands, may clarify further whether any hormonal mediation of hypervitaminosis A effects exists.

SUMMARY

The effects of hypervitaminosis A on the skeleton of weanling rats are accelerated by adrenalectomy, and no significant influence of cortisone on the process is evident.

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Muscle Necrosis Associated with Carbon Monoxide Poisoning

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Muscle necrosis associated with severe carbon monoxide poisoning is a relatively rare condition, possibly in part due to the fact that muscles are not examined during many of the routine autopsies performed in such cases. It appears, therefore, worth while to report the following case.

REPORT OF CASE

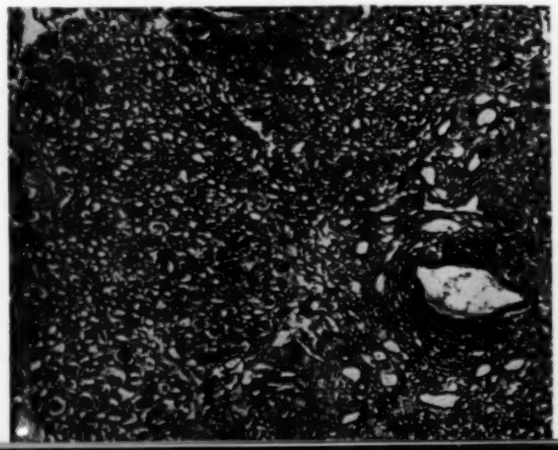
History.—A 35-year-old, muscular man was admitted to St. Joseph's Hospital at 12:30 p. m. on Sept. 7, 1954. He had returned to his home the evening before at about 11 p. m. and had attempted suicide by opening the gas jet. He was found by a friend shortly before admission to hospital and seemed to have regained consciousness after resuscitation. On admission to the hospital the patient appeared quite drowsy and physical examination showed a marked swelling of the right shoulder region and upper arm and thorax. There was also a small hematoma over the frontal region and a laceration across the bridge of his nose, indicating that he possibly fell forward during the carbon monoxide intoxication. Physical examination otherwise appeared not remarkable. On admission a brownish pigmented area was noted in the right axilla, which measured about 8×3 cm. in extent. There was, however, no evidence of vesiculation in this area. Subsequently, during his stay in the hospital the swelling in the right shoulder region increased considerably and involved the entire arm. The swelling was of brawny character. There was also some cyanosis of the finger tips. The radial pulse, however, was present during his entire stay in the hospital. It was at first thought that the patient had a deep hematoma, and an attempt was made to withdraw blood while a preparation of hyaluronidase was being injected into the pectoral muscle. There was no improvement following this treatment on Sept. 13. On Sept, 13, 14, and 15 procaine hydrochloride (Novocain) was injected into the right stellate ganglion, with ensuing vascular dilatation. There was, however, little change in the swelling of his arm. On Sept. 23 a muscle biopsy specimen was taken from the right pectoral muscle. The histological findings will be given below. On Sept. 24 the swelling gradually started to decrease and disappeared almost completely at the time of the discharge from the hospital on Sept. 28. There was, however, still marked firmness in the region of the pectoral muscle and marked limitation of movement. There was also tenderness of the pectoral and deltoid region at this time. The patient was last seen on Oct. 1, in the Outpatient Department, showing absence of swelling but marked weakness of the shoulder musculature.

Laboratory Findings.—Sept. 8: WBC, 17,750 per cubic millimeter. Urine, alkaline, straw-colored, and hazy. Albumin, negative. Sugar, 2+. The patient showed evidence of polyuria throughout the following day.

Sept. 11: NPN, 85 mg. per 100 cc.

Sept. 13: NPN, 80 mg. per 100 cc. Uric acid, 9.5 mg. per 100 cc. Creatinine, 3.8 mg. per 100 cc. NPN, uric acid, and creatinine returned to normal level on Sept. 16. Other laboratory findings were not relevant.

Fig. 1.—Necrotic muscle fibers, empty sarcolemma sheaths, and edema can be seen. On the right the necrotic artery is found surrounded by a cuff of inflammatory cells. Hematoxylin and eosin; × 47.



Submitted for publication March 8, 1955.

From the Department of Pathology, St. Joseph's Hospital.

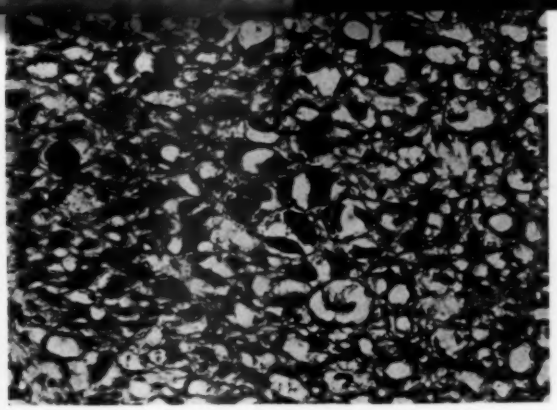


Fig. 2.—Necrotic muscle fibers, empty sarcolemma sheaths, and proliferating sarcolemma cells. Hematoxylin and eosin; × 134.

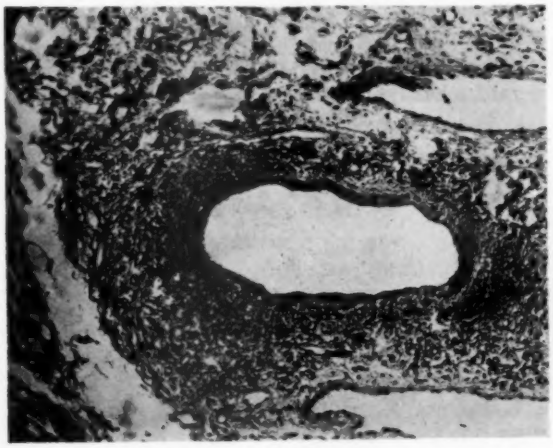
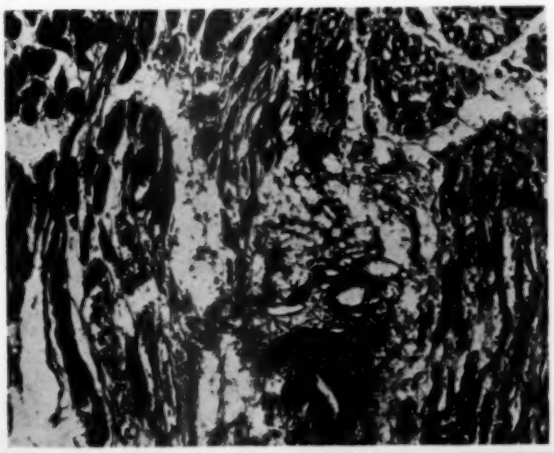


Fig. 3.—Necrotizing arteriolar lesion with surrounding cuff of mononuclear cells. Hematoxylin and eosin; × 134.

Pathological report of the biopsy specimen removed on Sept. 23 was as follows:

Gross: The specimen consists of a piece of muscle measuring 1×0.5×0.1 cm. It is reddish in color, soft in consistency, and appears to be ischemic.

Fig. 4.—Muscle necrosis, interstitial edema, and infiltration by mononuclear cells. Hematoxylin and eosin; × 134.



Microscopic: Sections from different areas of muscle show necrotic muscle fibers with numerous sarcolemma sheaths which show absence of muscle tissue. In some areas there is proliferation of the sarcolemma cells. The muscle fibers in other areas show loss of striation, waxy and hyaline-like degeneration, and fragmentation, and in other areas evidence of shrinkage. The interstitial tissue shows quite extensive infiltration by mononuclear cells and, particularly, histiocytes; some of the small arteries show evidence of fibrinoid necrosis of the intima and subintima with extensive infiltration by polymorphonuclear cells, eosinophiles, and mononuclear cells which extend into the adventitia. On several sections there was no evidence of hemorrhage. In some of the peripheral areas of the section examined, there was also evidence of calcification. In summary, the process was that of muscle necrosis involving also some of the blood vessels with associated sarcolemma proliferation. This process most likely was associated with carbon monoxide poisoning.

COMMENT

The occurrence of muscle necrosis in cases of severe carbon monoxide poisoning is rare but has been recognized for a long time. The first observation was by Larrey,[1] Napoleon's physician, on a group of French soldiers who died from carbon monoxide poisoning while in barracks in Berlin in 1806. He describes necrosis of the muscle over sacrum and scapulas. Hedinger [2] has reviewed the literature exhaustively and has found 12 cases verified by biopsy or autopsy. He has reported two cases of his own. The clinical picture in most of these cases is very similar to our own case, the brawny type of edema being the most striking feature. This process is frequently accompanied by changes in the overlying skin, such as erythema, vesiculation, necrosis, or gangrene.

The end-result of this condition at times is one of muscle contracture of the Volkmann type. The pathological findings in the 12 histologically corroborated cases vary from degenerative changes (fatty, hyaline, or waxy) to necrosis and calcification, even after a few

days from onset of the disease condition. Vascular occlusions have been shown in only a few cases, and changes in the arteries as found in our own case have not been described. It appears, however, that these changes are quite compatible with a localized necrotizing process. There certainly was no evidence of generalized arteriolar disease in this patient. The onset of muscle damage throughout the various reports has varied from the moment the patient has regained consciousness to apparently 10 weeks after the poisoning.

The pathogenesis of muscle necrosis has in the past led to considerable difference of opinion which is well described in Hedinger's article.[2] Some authors think that it is a mere mechanical phenomenon and carbon monoxide poisoning a mere coincidence; others take opposite views. Adams, Denny-Brown, and Pearson [3] take an interesting view of the problem which seems very convincing to me. The mechanical component in muscle necrosis is certainly of primary importance, shown also by localized occurrence of necrosis in isolated muscle groups only and its frequent association with localized skin changes. Pressure certainly leads to ischemia. It appears, however, that associated anoxemia due to carbon monoxide poisoning with reduction of oxygen saturation of blood is necessary to cause necrosis; otherwise mechanical muscle necrosis would be considerably more frequent. Another interesting point mentioned by the above authors [3] is that carbon monoxide poisoning possibly leads to interference with the oxidative enzymes of muscle tissue by forming carbon monoxide myoglobin. Another factor in favor of functional damage to muscle opposed to mechanical factors is the occurrence of myocardial necrosis which has been described by many authors.*

An interesting incidental finding in our case was the occurrence of temporary oliguria and azotemia, most likely secondary to myoglobinuria following massive muscle destruction. Unfortunately, we were not aware of the nature of the patient's condition at the time, and the urine was not examined spectroscopically.

SUMMARY

A case of muscle necrosis in the pectoral region occurring in a young man who attempted suicide by carbon monoxide poisoning has been described, and the pertaining literature has been briefly discussed.

Dr. E. B. Cahoon and Dr. E. C. Grundy granted permission to publish this case. Dr. D. H. Bowden, Sick Childrens Hospital, reviewed the biopsy material and pointed out a most valuable reference.

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Neuropathology of Rats Experimentally Poisoned with p-Tertiary-Butyltoluene

Investigations with Consideration of So-Called Mucoid Change of Nerve Tissue

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Previous investigations in this laboratory have suggested that histologic lesions of the white matter in the brain of laboratory rats may be produced by the inhalation of an aromatic hydrocarbon, *p*-tertiary-butyltoluene (TBT).[1] In certain animals thus exposed, the lesions were accompanied by the appearance of metachromatic mucicarminophilic material, e. g., mucopolysaccharides or glycoproteins. The significance of such substances in lesions of the central nervous system has been a controversial issue. Some authors have related it to demyelinating diseases in human beings.[2] Others have discussed it as a possible artifact, in view of the occasional finding of "mucoid matter" in "normal" human brains [3] as well as in a large number of human diseases and terminal stages of disease.[4] Finally, attempts to reproduce such lesions by variations in the procedures of fixation or embedding have remained inconclusive.*

The present experiments were continued to study the metachromatic material which appears in the central nervous system following poisoning by TBT. In addition, it was deemed appropriate to describe the pathology of TBT poisoning, since it is somewhat similar to the lower aromatics in pharmacologic action. Reports of poisoning by benzene and some of its derivatives have recorded severe neurologic symptoms but have not described in detail the morphologic changes in the central nervous system.†

EXPERIMENTAL PROCEDURE

Conditions of Exposure.—Groups of female rats of the Long-Evans strain, weighing 150 to 200 gm. and maintained on a standard laboratory diet, were given single or multiple exposures to the vapors of TBT. The technique has been described previously.[1] In order to eliminate lesions resulting from agonal changes, tissues for these microscopic studies were taken only from animals which survived. The rats included in the present work were given exposures as follows.

Single Exposures: Three rats exposed to 670 ppm for two hours and two exposed to 1000 ppm for 45 minutes were killed four hours later. Two rats exposed to 670 ppm for two hours and three exposed to 1000 ppm were killed 22 hours later. Four rats exposed to 400 ppm until severely intoxicated (two to three hours) were killed after 22 hours.

In order to study the phenomena of recovery, 12 rats were exposed to 400 ppm and removed from the chamber between the second and third hours, as signs of intoxication became pronounced.

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From the Department of Pharmacology and Experimental Therapeutics and the Department of Pathology, University of California Medical School, San Francisco, and the Department of Pathology, Hebrew University—Hadassah Medical School, Jerusalem.

* References 5 and 6.

† References 7 through 10.

Groups of four were killed 7, 14, and 28 days after exposure. Four rats from groups exposed to 200 ppm for four or eight hours or to 670 ppm for one hour and killed 10 days later were included in the study, since they had shown clinical evidence of severe intoxication including paralysis of the forelegs or hind legs. Data concerning the rats in this group are summarized in the accompanying Table.

Multiple Exposure: Groups of rats were given 25 and 127 exposures to 50 ppm and 50 and 127 exposures to 25 ppm. These concentrations had been found to be innocuous on single inhalation.[1] Each of the groups was divided into four subgroups, exposed for one, two, four, or seven hours daily except week ends. Each subgroup contained four or five rats, and all were killed 24 hours after the final exposure.

Control Rats: A randomly selected group of 35 control rats was examined to evaluate technical artifacts and to exclude the possibility of clinically unrecognized mild infections of the central nervous system in the stock.

Preparation of Tissues.—After decapitation of the rats, portions of brain, spinal cord at several levels, and sciatic nerves were fixed in 10% formaldehyde and in Carnoy's fluid (absolute alcohol with chloroform and acetic acid in proportions of 6:3:1). Paraffin sections were stained with hematoxylin and eosin, Weil-Lillie's method for myelin, Mallory's phosphotungstic hematoxylin, 0.5% toluidine blue, Mayer's mucicarmine, McManus's periodic acid-Schiff procedure, and the colloidal iron method.[11] Marchi preparations were also made from brain and cord of the 16 acutely poisoned rats, which were observed for late effects.

Solubility tests were performed in an attempt to identify metachromatic mucicarminophilic matter in the cytoplasm and in vacuoles.

FINDINGS

The principal toxicologic responses to vapor exposure were impairment of the central nervous system and of respiratory function. Disturbances in muscle tone and activity were most striking; they included retrograde movements, irregular gait, and paralysis of the spastic or flaccid type. Survivors with neurologic signs did not always recover completely during the course of the experiment. Respiratory disturbances ranged from slight nasal discharge to profuse discharge, salivation, and extreme respiratory embarrassment due to pulmonary effusion.

Single Exposure.—Acute Changes: All of the animals exposed to from 200 to 1000 ppm for from 45 minutes to eight hours showed similar changes in the central nervous system.

Metachromatic material was found in glial elements and in relation to myelin sheaths in the cord three hours after exposure. The affected glial elements were predominantly oligodendrocytes and were found in the cerebrum along the lateral ventricles, extending toward the cortex, and in the corpus callosum. The metachromatic material was present in the cytoplasm and stained positively with mucicarmine (Fig. 1), periodic acid-Schiff, and colloidal iron. It did not stain with Weil-Lillie's method for myelin. It could not be determined whether the metachromatic material was within or between the myelin sheaths in the cord.

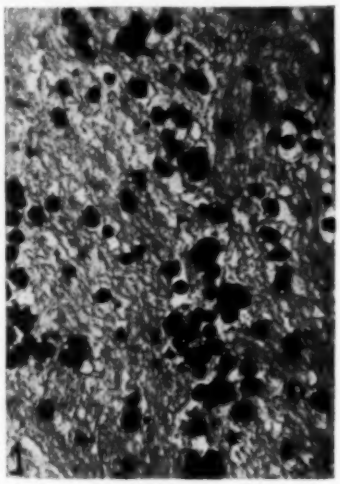


Fig. 1.—Four hours after single exposure to 670 ppm for two hours. Cerebrum, near corpus callosum. Gliocytes with accumulation of metachromatic substance. Hematoxylin mucicarmine; ×370.

The quantity of metachromatic material contained in vacuoles increased with increasing time after exposure (Fig. 2). The vacuoles were scattered throughout the white matter, the corpus callosum, medulla oblongata, cerebellum, and cord. In the cord they were also found in the gray matter, adjacent to nerve cells or displacing them.

The appearance and increase of metachromatic substance in the central nervous

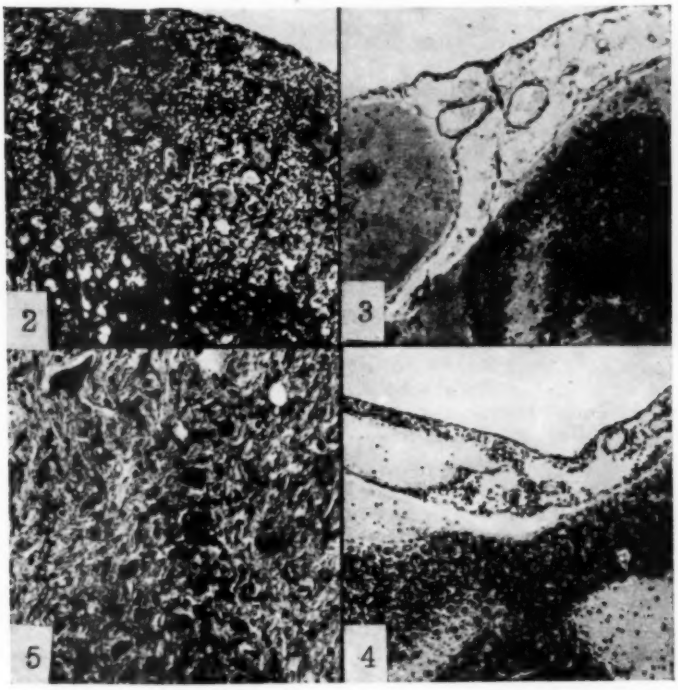


Fig. 2.—Seven hours after exposure to 670 ppm for one hour. Cord with edema, vacuolation, and neuronophagia. Hematoxlin-eosin; × 72.

Fig. 3.—Same exposure as Figure 2. Edema of meninges. Hematoxlyin-eosin; × 72.

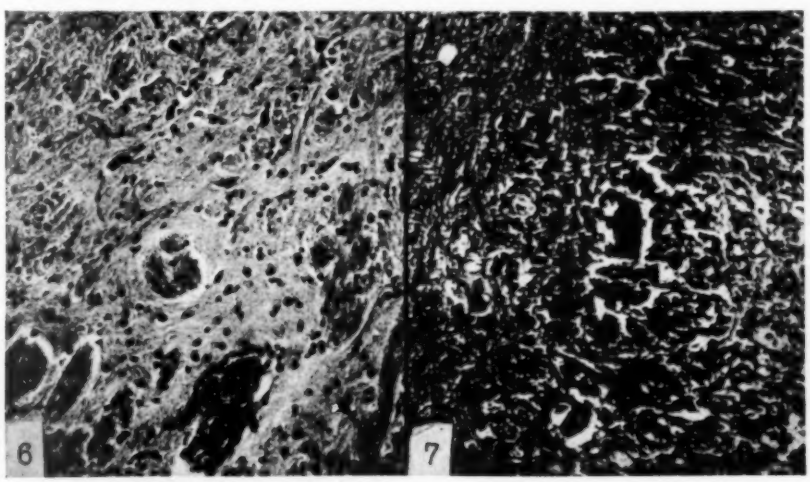
Fig. 4. — Twenty-two hours after exposure to 1000 ppm for one hour. Edema and round-cell infiltration in meninges. Hematoxylin-eosin; × 72.

Fig. 5.—Same exposure as Figure 4. Striatum, severe neuronal damage. Glia-cell foci in areas of neuronophagia. Hematoxylin-eosin; × 160.

system was accompanied by other alterations. There was meningeal edema, which became severer with increasing intervals of time after exposure to TBT (Fig. 3). After a period of 22 hours the meninges were markedly infiltrated by small round cells (Fig. 4). Neuronal swelling, satellitosis, and occasional neuronophagia were present in the cerebral cortex, striatum, and hippocampus (Fig. 5). Chromatolysis and nuclear

Fig. 6.—Same exposure as Figure 4. Perivascular foci of small round cells in cerebrum. Hematoxylin-eosin; × 160.

Fig. 7.—Twenty-two hours after exposure to 400 ppm for three hours. Perivascular necrosis in medulla oblongata and glial invasion. The central area of the focus contains metachromatic matter. Periodic acid-Schiff method; × 144.



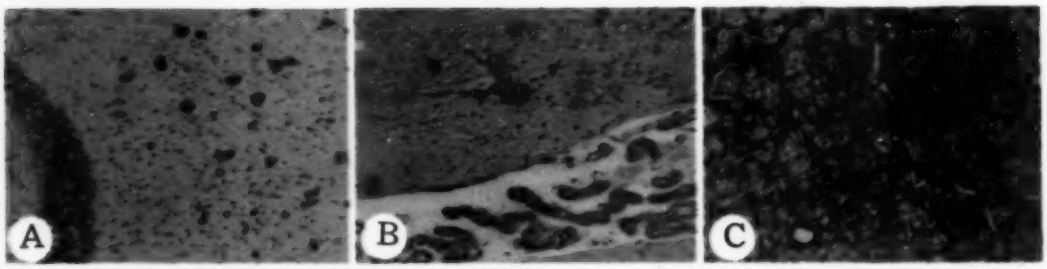


Fig. 8.—Vacuoles containing metachromatic matter nine to ten days after single exposure.
A, exposure to 670 ppm for one hour. Toluidine blue; × 72.
B, exposure to 200 ppm for eight hours. Periodic acid-Schiff; × 72.
C, exposure to 200 ppm for four hours. Colloidal iron; × 72.
The color micrograph was made by Mr. H. R. Luck, Photographic Department, Shell Development Laboratories, Emeryville, Calif.

vacuolation of neurons were occasionally prominent in the anterior gray columns of the cord. These latter changes were severer 22 hours after exposure, and at this time acute focal necrosis was seen in the white matter, particularly in the cord; occasionally, infiltrations of small round cells occurred around blood vessels (Fig. 6). The centers of necrotic foci often contained metachromatic material and were invaded by glial elements (Fig. 7).

There were no alterations in the sciatic nerves in any of the animals of this group.

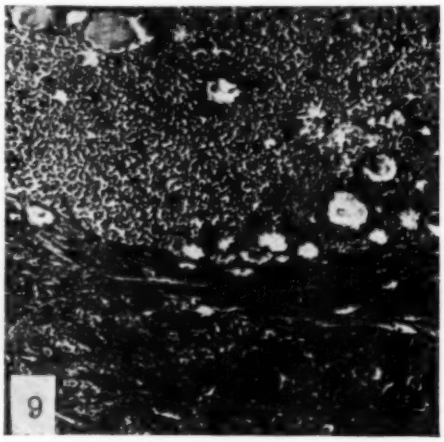
Late Changes: The pathologic changes in the central nervous system were essentially the same, whether 7, 10, or 14 days after a single exposure. Vacuoles containing metachromatic material were few in number and localized. In those rats exposed to a high concentration of TBT for even as short a period of time as one hour and those exposed to a low concentration for eight hours, the vacuoles were widely distributed in the brain, cerebellum, and cord (Figs. 8 and 9).

At 28 days after exposure, only a few rare and isolated vacuoles of metachromatic substance were found in the corpus callosum, similar in quantity to those found in normal control rats.

At all stages there was, in addition, shrinkage and occasionally even destruction of neurons in the rats most severely affected. Satellitosis, chiefly of swollen oligodendrocytes, was present together with diffuse glial increase, particularly in areas of neuronal destruction (Fig. 10). Glial nodules occurred in the white matter of the medulla oblongata (Fig. 11) and gray matter of the cord, with mild edema of the white matter of the cord. Twenty-eight days after exposure, vestiges of the previous damage were visible. There was patchy loss of nerve cells, occasional satellitosis, and neuronal shrinkage in the lower and middle layers of the cortex, and moderate widespread increase of glial cells; foci of small round cells were present around vessels or isolated neurons showing chronic damage (Fig. 12).

In the brain, patchy loss of myelin was evident in the corpus callosum, lamina quad-

Fig. 9.—Eleven days after exposure to 200 ppm for four hours. Glial nodule in gray commissure of cord. Vacuolar change containing metachromatic material in white matter. Hematoxylin-eosin; × 144.



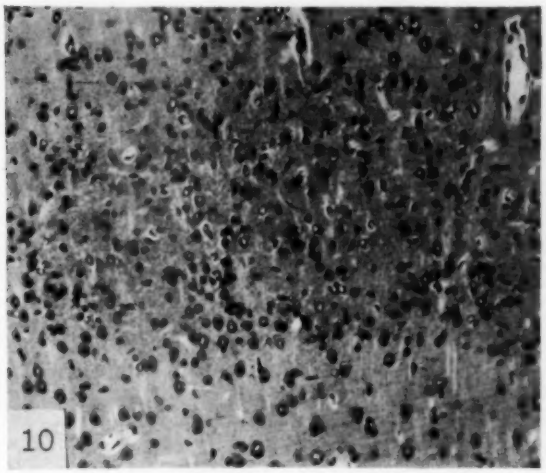


Fig. 10.—Same exposure as Figure 8. Neuron destruction in cerebral cortex; glia-cell reaction. Hematoxylin-eosin; × 144.

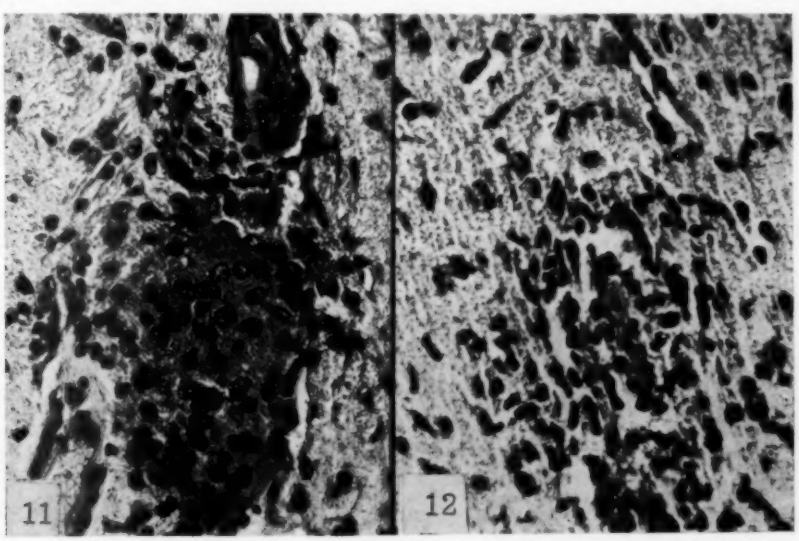
rigemina, and brachia pontis. In some areas, demyelination was found surrounding foci of small round cells. Transverse and longitudinal sections through the spinal cords of all four animals revealed myelin loss in the peripheral areas to a varying extent at the different levels examined (Fig. 13).

At 28 days after exposure, the sciatic nerves of three of four rats showed areas of demyelination of scattered fibers, which in the rats with paralysis were partly replaced by fibrosis. The brachial plexus from one animal with paralyzed forelegs showed edema in the peri- and epineurium and partial demyelination and axon destruction. Occasional proliferation of endoneural nuclei, fibrosis, and thickened small blood vessels were observed (Fig. 14).

Marchi preparations at 28 days after exposure showed in the cords of the paralyzed

Fig. 11.—Two weeks after exposure to 400 ppm for three hours; the rat had paralysis of hind legs. Glia-cell nodule in medulla oblongata. Hematoxylin-eosin; × 450.

Fig. 12.—Twenty-eight days after exposure to 400 ppm for three hours. Focus of gliosis. Periodic-acid-Schiff method; × 400.



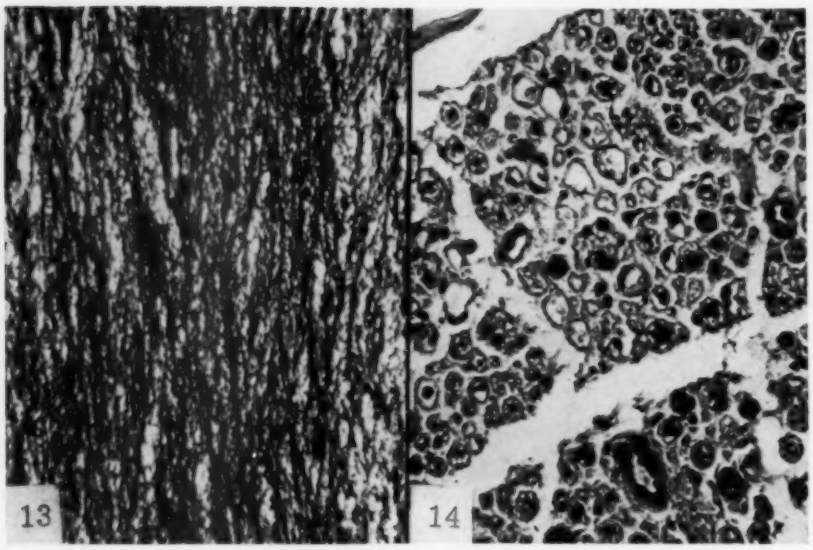


Fig. 13.—Same exposure as Figure 12. Longitudinal section through white matter of thoracic cord, showing areas of demyelination. Weil-Lillie's method for myelin; × 120.

Fig. 14.—Same exposure as Figure 12. Brachial plexus of rat with persistent paralysis of forelegs. Severe secondary degeneration. Periodic acid-Schiff method; × 400.

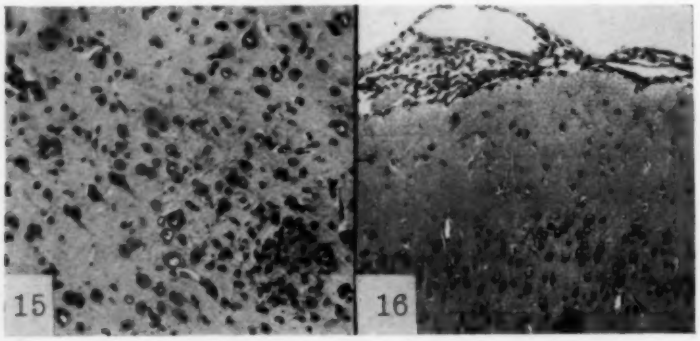
rats numerous degenerated fibers throughout but predominantly in the anterior and anterolateral tracts. Sciatic nerves were free of changes, but the brachial plexus showed marked degeneration and numerous "Marchi-balls."

Repeated Exposure.—Exposure to 50 Ppm: In the group of 17 rats given 25 exposures over a period of five weeks, those exposed for seven-hour periods showed moderate perivascular edema. The same lesion was found to a milder degree in rats exposed for four-hour periods and could hardly be detected in three of four rats in the groups exposed for two- and one-hour periods.

In each of the four groups, one rat presented accumulations of small round cells around blood vessels in the cortex and pons, and, occasionally, focal glial cell proliferation (Fig. 15). The same animals had meningeal

Fig. 15.—Twenty-four hours after 25 exposures to 50 ppm for one hour daily. In white matter of frontal lobe, perivascular accumulations of small round cells and glia-cell foci. Hematoxylin-eosin; × 144.

Fig. 16.—Twenty-four hours after 25 exposures to 50 ppm for four hours daily. Meningeal infiltration with mononuclear cells. Hematoxylin-eosin; × 144.



infiltration with small round cells and histiocytoid elements (Fig. 16). Myelin stains revealed irregular small areas of myelin loss in the corpus callosum, pedunculi cerebelli, and/or white matter of the cord peripherally.

In the group of 18 rats given 127 exposures over a period of six months, only one of the four exposed for seven hours revealed changes in the nerve tissue comparable in character and quantity to those found after 26 exposures. In this animal, at various levels of the cord, the white matter, especially in the posterior columns, contained scattered accumulations of glia cells. Some of these showed excentric small nuclei, and the cytoplasm contained metachromatic substance. In one level of the thoracic portion, the gray matter was completely replaced by gliosis (Fig. 17). In the four-hour group, two of five rats had small glia-cell foci in the cerebellum and marked satellitosis in the thalamic nuclei. No pathologic changes were present in the groups exposed for one and two hours.

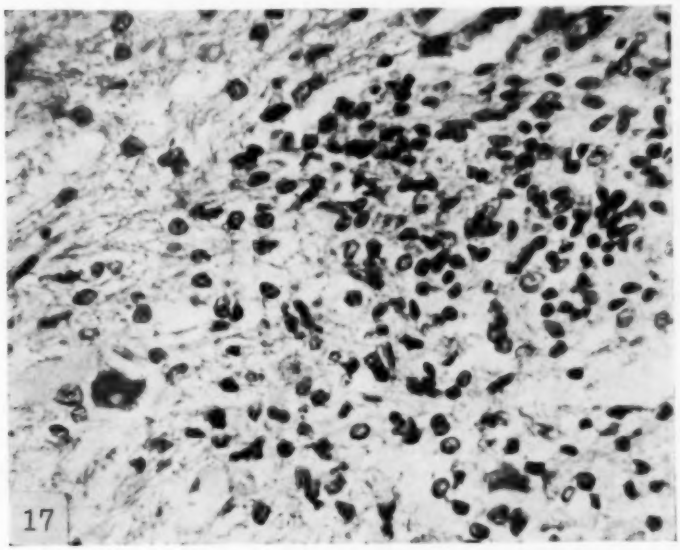


Fig. 17.—Twenty-four hours after 127 exposures to 50 ppm for seven hours daily. Thoracic cord. Total replacement of gray matter by gliosis; only a few neurons were preserved. Periodic acid-Schiff method; × 450.

The myelin stain revealed changes no more advanced than in the shorter experiments, while the sciatic nerves revealed no changes in the animals of this series.

Exposure to 25 Ppm: Fifty exposures were given to 18 rats over a period of 10 weeks, and 127 exposures to 26 rats over a period of six months. One rat each in the seven-hour, four-hour, and two-hour groups showed lesions essentially similar to those in the preceding series. The rat in the two-hour group had, in addition, several glia-cell nodules throughout the white matter and perivascular accumulations of small round cells in the cortex and medulla oblongata. No changes were observed in animals exposed for one hour daily.

The sciatic nerves showed no changes at any exposure to 25 ppm.

Control Rats.—In 35 randomly selected control rats exposed to air uncontaminated with TBT, none of the lesions described above were observed. However, in several rats, small metachromatic vacuoles were seen in the corpus callosum and in the white matter of the cerebellum. No metachromatic matter was seen within the cytoplasm of glia cells in any of the controls.

Solubility Tests.—Fixation in Carnoy's solution followed by paraffin embedding through benzene or toluene at room temperature dissolved entirely the myelin sheath lipids but preserved the metachromatic material in vacuoles and in cytoplasm of glia cells. Likewise, the staining reactions were

almost undiminished after extraction with ether for 18 hours at 37 C.

The metachromatic material was dissolved after 6 hours at room temperature in pyridine, and after 6 hours in acetone and 18 hours in Carnoy's mixture or equal parts of methanol and chloroform at 37 C.

COMMENT

General Considerations.—The lesions described in this article permit the classification of TBT among poisons of the central nervous system affecting primarily the white matter, the neuronal lesions being irregular and of less extent. Glial reaction was observed diffusely or appeared as focal cell accumulations at the sites of demyelination or neuronal destruction. This type of lesion was regularly present following acute poisoning, while in animals exposed repeatedly to nontoxic doses variations in individual sensitivity of animals appeared of more significance than the length of the experiment or the time of exposure.

Peripheral nerve lesions were observed only during the phase of acute poisoning and were related to the severity of damage to the central nervous system, and it is thus unlikely that they represented a direct effect of TBT poisoning.

It is unlikely that the predominant effect of TBT on the white matter is due to its character as a lipid solvent, since similar lesions have been shown to result from poisoning with nonsolvents as well; for example, in poisoning by hydrogen phosphide,[12] carbon monoxide, azide, malononitrile, and cyanide.[13]

In our material, a primary vascular damage seemed more likely, as evidenced in the earlier stages by edema within the meninges and around blood vessels, followed later by round-cell infiltrations in the same areas, and by the generally diffuse distribution of the lesion, which was sometimes more intense around the blood vessels. This would agree with observations of marked edema and purpura-like hemorrhages in the brain in cases of acute and subacute benzene and toluene poisoning.[8]

Metachromatic Change.—In rats poisoned with TBT, metachromatic-mucicarminophilic material was observed in the white matter of the central nervous system. The amount appeared to be dependent, first, on the intensity of poisoning and, second, on the interval following it; it increased during the first 22 hours and had decreased by the 14th day subsequent to a single exposure to toxic vapors. In other animals, exposed repeatedly to nontoxic concentrations of TBT, metachromatic vacuoles were seen in small numbers and were no more abundant than in the control animals.

It cannot be disproved that the structure of the large metachromatic vacuoles in the later stages of acute poisoning is the result of unknown effects of fixation and embedding procedures, as suggested by Bielschowsky [3] and Weil.[14] However, this does not eliminate their pathological significance, in view of their quantitative relationship to the time lapse after exposure and to the intensity of poisoning. Our observations appear to support those investigators who concluded, from the study of autopsy material, that the vacuolar lesions containing mucicarminophilic matter may represent "useful artifacts" revealing lesions which otherwise could not be demonstrated, such as an alteration in the composition of myelin or ground substance, occurring before death.‡

This view is further supported by the finding of metachromatic material within the cytoplasm of glial elements as one of the earliest changes in animals acutely poisoned, as well as in some of the animals subjected to repeated small doses of TBT over prolonged periods of time. The appearance of intracellular metachromatic material before vacuoles were formed seems to exclude the possibility that the metachromatic change of the gliocyte is the result of phagocytosis. Were this the case, we should expect a greater number of metachromatic glia cells during the period of recovery. This, however, did not occur. Glial nodules following acute poisoning were more frequent at the

‡ References 5 and 6.

later periods of observation and, at four weeks, surrounding areas of demyelination or of neuronal destruction were sometimes seen, their cells being free of metachromatic matter. The above findings appear, therefore, to support the idea of a metabolic relationship between myelin sheaths and oligodendroglia, whereby degenerative changes in the latter will impair the former.[2]

The Nature of the Metachromatic Material.—An attempt was made to identify the metachromatic material by its solubility and its staining reactions.

Anhydric processing of Carnoy-fixed material followed by extraction by pyridine produced entirely negative staining results, making it unlikely that the compound is essentially a water-soluble one.

The relative resistance to cold solvents, such as Carnoy's mixture and solvents used during the processing of tissues for paraffin embedding, and the easy solubility in pyridine made it likely that the metachromatic material belonged to the group of cerebrosides.[15] This probability is further supported by its relative resistance to ether extraction and negative staining after treatment with acetone, which dissolves all brain lipids with the exception of phosphatides.[16]

Among fatty substances, unsaturated lipids, sphingomyelin, and cerebrosides have been shown to be revealed by the periodic acid-Schiff procedure.[17] The above experiments with solvents demonstrate that only the last group need be considered here.

Metachromasia may be observed in mixtures of, but not in all, cerebrosides.[18] Thus, for example, the cerebrosides of Gaucher cells in the spleen, after fixation with Carnoy's fluid, stained bright red with the periodic acid-Schiff method but did not react with toluidine blue or by any of the other methods of staining that revealed metachromasia in the present material (unpublished observations). Following the discovery by Blix of a sulfuric ester formed with the primary alcoholic hydroxyl group of the sugar portion of brain cerebrosides,[19] Wislocki and Singer suggested that metachromasia of normal myelin may be dependent on the presence of sulfatides. After elimination of phosphatides by solution, the occurrence of a sulfated cerebroside as the main substance in the material studied by us seems possible. However, it cannot be considered to be identical with the metachromatic substance observed in frozen sections of the "normal" myelin sheath,§ which dissolves in cold alcohol and cold benzene during the embedding procedure.

This conclusion is in agreement with the hypothetical explanation of the colloidal iron procedure used. This method has been variously considered to be a specific stain for acid polysaccharides ‖ or to be devoid of all histochemical significance.¶ The perfect agreement in our study between the colloidal iron method and the metachromatic staining reaction may support the view that it is an acid radical attached to the hexose moiety of cerebroside, which determined the positive result with this method.

The observation that the metachromatic matter reacted also with mucicarmine deserves consideration. The specificity of this method for mucins was doubted by Sylvén,[26] who showed that a positive stain may be obtained in any tissue elements containing higher sulfuric acid esters. Feyrter[27] observed staining of Gaucher cells with mucicarmine, referring the results to reaction of the aluminum carminate with the carbohydrate portion of cerebroside. These facts appear to support the view expressed by Smith[6] that "metachromatic changes" in central nerve tissue signify lesions identical with those referred to in the literature as "mucocytic degeneration of the oligodendroglia."[28] This conclusion is supported in our material by morphologic similarity of the metachromatic vacuoles to the lesion referred to as "mucocytes."

SUMMARY

Inhalation of vapors of *p*-tertiary-butyltoluene (TBT) produced lesions in the cen-

§ References 20 through 22.

‖ References 23 and 11.

¶ References 24 and 25.

Neurologic Signs During Recovery of Rats from Acute TBT Poisoning*

Time, in Days, from End of Exposure to Necropsy	No. of Rats	Concentration of Vapor, Ppm	Duration of Exposure, Hr.	Clinical Observations
10	1	200	4	Transient paralysis of forelegs
10	1	200	8	Transient paralysis of hind legs
10	2	670	1	Transient paralysis of hind legs
7	4	400	3	2 had paralyzed hind legs on 2d to 5th or 6th day
14	4	400	3	2 had transient paralysis of hind legs
28	4	400	3	1 had transient paralysis of hind legs; another had persistent paralysis of forelegs

* The rats exposed to 200 and 670 ppm were selected from the acute toxicity experiment [1] as suitable for this study. Rats exposed to 400 ppm were exposed especially for histologic study and were not included in the previous paper.

tral nervous system of rats. The lesions following acute poisoning were characterized by edema of the meninges and white matter, myelin degeneration, and reactive gliosis. Metachromatic-mucicarminophilic material, which at the earliest stage of poisoning was observed within the cytoplasm of glia cells, eventually was seen only within large, poorly defined, vacuoles. The conclusion was reached that these changes were of biologic significance, although the possibility was considered that in later stages the vacuoles may be the result of artifacts produced by fixation.

Protracted poisoning by multiple exposure to low concentrations of TBT resulted only occasionally in focal demyelination and gliosis. Similar lesions were observed after recovery from acute poisoning, when the metachromatic change had also regressed.

The nature of the metachromatic material was investigated with regard to staining properties and behavior toward various solvents. It appears to be different from myelin, is probably a cerebroside, and may be a sulfatide.

The micrographs, Figures 1, 6, 8, 11, 14, and 17, are the work of Mrs. H. Weinmann, Jerusalem; the remaining figures, that of Mr. H. R. Luck, Emeryville, Calif.

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The Pathology of Perinatal Distress

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A program for the systematic investigation of perinatal pathology was established in 1947 with the financial and technical assistance of various organizations. It was directed primarily at the common, yet little understood, lesions of stillborn and newborn infants rather than the unusual ones. The following is a summary of this work.

Among those pathological lesions which were frequently encountered, some had an obvious relationship to perinatal anoxia. Others could not be satisfactorily interpreted until it became apparent that they occur particularly in those infants who have a history as well as known pathological signs of perinatal distress. Eventually, all common pathological lesions of newborn infants appeared to be interrelated parts of what will here be called the "perinatal distress syndrome," with the exception of the direct effects of mechanical trauma, or such conditions unrelated to birth as, for instance, erythroblastosis, malformations, or intrauterine infections. The perinatal distress syndrome comprises pathologic changes due to the effects of anoxia and shock. Each of these changes, taken separately, is nonspecific as to the cause of distress, as are similar lesions in the adult. Some characteristic features of the syndrome are due to peculiarities of the newborn infant or to the association of several lesions. As was pointed out in a preliminary report,[1] some of these changes are direct effects of anoxia and others are identical with those known to occur as "shock lesions." The association of asphyxia and shock is to be expected in view of the very close relationship of the two and the fact that both may jointly contribute to the establishment of a vicious circle.[2] For this same reason it is impossible to separate the functional and morphologic changes due to asphyxia from those caused by shock. Either or both of these factors are responsible for the various lesions comprising the distress syndrome. Based on evidence of various kinds, other authors have suggested that newborn infants may be in shock; their work will be mentioned in the discussion of the present findings.

Many disorders may bring about distress of the fetus or infant about the time of birth. The causative factor may reside outside the fetus and then cannot be determined at autopsy. Detailed observation of the mother and, as far as possible, the fetus during labor will indicate cause and duration of intrauterine distress in some instances; in other cases only the effects upon the fetus are apparent. Whether or not the course of events leading to distress is known, one is presented with an infant exhibiting some degree of the pathologic distress syndrome and associated clinical signs and symptoms. These pathologic and clinical manifestations are nonspecific, as far as the cause of distress is concerned, and must be treated regardless of their individual extrinsic causes. This justifies a description and discussion of the pathologic syndrome, even though the pre-

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ceding history may not be adequately known in many cases. The importance of knowledge of the distress syndrome is emphasized by the fact that neonatal mortality has not been lowered to a great extent in recent years, certainly less than maternal mortality. Not only may such knowledge be useful in the proper interpretation of individual cases of perinatal deaths, but it may point the way to new methods of reducing neonatal mortality.

The present summary of the pathologic findings and related statistical data should serve to present a rounded concept of the subject of which, so far, only some outstanding points have been published.

MATERIAL AND METHODS

The material of the Brooklyn Survey of Neonatal Pathology is derived from various sources. The largest single group of cases was obtained from autopsies performed at Kings County Hospital by me or under my supervision. Other hospitals in Brooklyn furnished preserved tissues with copies of their autopsy protocol and an abstract of the history.

Among the first 1000 consecutive autopsies listed in the Brooklyn Survey of Neonatal Pathology there were 705 stillborn and newborn infants up to the age of 3 days. Of these, 137 were excluded because of severe intrauterine maceration, and 68 because of severe malformations or prenatal disease which would have made proper function of vital mechanisms impossible even under favorable circumstances. Examples of the latter are infants with polycystic kidneys and hypoplastic lungs, or those with severe manifestations of congenital syphilis. Also excluded were 48 instances in which pathologic information was inadequate; the criteria will be explained later. Thus, 452 cases remained to be included in the analysis.

Tissues obtained at autopsy were fixed in Bouin's fluid and stained with the trichrome method of Gomori.[3] Other stains were used as needed. The number of tissue blocks from complete autopsies varied usually from 15 to 25. Frozen sections were occasionally prepared and stained with Sudan III or IV and hematoxylin.

For the statistical evaluation of signs of asphyxia and shock, a card was made out for each case, listing the changes enumerated below. For the analysis shown in Figure 9, arbitrary scores were obtained by allotting to various lesions the maximum values or fractions thereof listed in Table 1.

TABLE 1.—*Data of Maximum Values Allotted to Various Lesions to Obtain Scores of Pathologic Changes Referable to Perinatal Distress*

Edema (except of presenting part)	1
Petechiae (except those separately listed)	1
Dilatation of the heart	2
Necrosis in arteries (heart, liver)	2
Aspiration of amniotic sac contents	2
Bronchopneumonia	2
Degeneration of liver cells	3
Pancreatic lesions	2
Adrenal hemorrhage, necrosis	3
Internal genitalia (severe hyperemia, hemorrhage)	1
Digestive tract (ulceration)	3
Brain and meninges (focal, presumable non-traumatic hemorrhage)	3

Other changes noted for each case, but not included in the numerical scores, were hyaline membranes in the lungs and mechanical intracranial birth injury.

In all instances the score was the number of positive points divided by the total number of points on which information (positive or negative) was available, multiplied by 100. Thus, in a case in which 8 of 25 possible points were positive, the final score would be $8/25 \times 100$, or 32. In a case in which 4 points were positive among 16 points examined, the score would be 25. In order to eliminate instances of very incomplete information, only those cases were included in which information (positive or negative) equivalent to 12 points was available. All data are arranged in weight groups of multiples of 500 gm. of body weight. No infants or fetuses weighing less than 200 gm. are included.

OBSERVATIONS

Only those pathological changes will be described in detail which have not been the subject of previous reports. Their relationship to distress may not be immediately apparent; this will be discussed in a subsequent section.

Cardiovascular System.—The cardiovascular system is prominently concerned in most of the changes to be described. There is congestion of blood vessels and capillary damage resulting in edema and hemorrhage. The heart may suffer when this occurs in its tissues, and this may in turn aggravate the damage to peripheral circulation. At first, edema, hemorrhage, and cardiac changes will be discussed; however, it should be kept in mind that in the pathogenesis of some other lesions, which will subsequently be described, damaged capillaries and impaired circulation are also important factors.

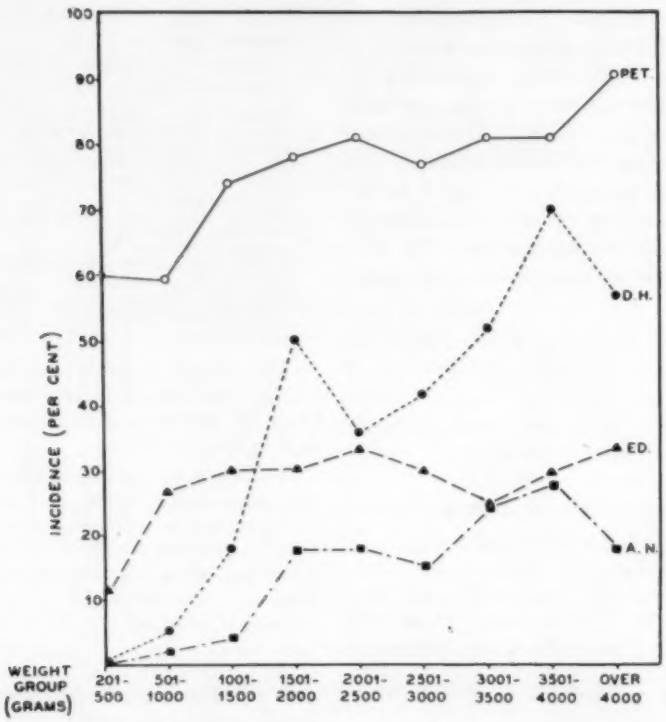

Fig. 1.—Incidence of lesions due to cardiovascular damage by weight groups. *A. N.*, arterial necrosis; *D. H.*, dilatation of the heart suggestive of myocardial damage; *ED.*, edema; *PET.*, petechiae.

Edema is often present to a conspicuous degree at birth. It may be prominent either in various portions of the subcutaneous tissue or internally, and, therefore, its external appearance varies widely. Hydrops is merely a severe degree of edema. Potter and Adair [4] have emphasized the great variability in its localization. Figure 1 shows that the incidence of edema in newborn infants (exclusive of that in the presenting parts) increases with body size, but in the viable groups (over 500 gm.) this increase is slight compared with other lesions. It is known that premature infants have a marked tendency to edema formation, apparently due to peculiarities of their electrolyte balance.[5] This may offset a lower incidence of edema due to distress in premature infants.

Focal hemorrhages are a well-known sign of asphyxia. They occur in almost any part of the body, but most frequently and intensively in the thoracic viscera, notably the epicardium, pleura, lungs, thymus, and esophagus. The most plausible explanation is that respiratory movements, particularly futile ones occurring without expansion of the lungs, produce negative pressure which enhances the extravasation of blood, much as it happens in tests using suction to examine the fragility of capillaries in the skin.

Focal hemorrhage in the adrenals, genital organs, digestive tract, and central nervous system will be described later in conjunction with more detailed accounts of changes in these organs. Other locations of focal hemorrhage are in the skin, peritoneum, and kidneys. In several infants the temporal muscles were diffusely hemorrhagic. The incidence of focal hemorrhages is given by weight groups in Figure 1. Its rate is the highest of all lesions tabulated and rises with body weight approximately in the same manner as do other manifestations.

Two types of lesions were encountered in the heart, namely, degeneration of the myocardium and focal necrosis in the wall of coronary arteries. The former manifests itself at autopsy by dilatation of the chambers of the heart, including both ventricles. This diagnosis is based on the pathologist's judgment at the time of dissection. In order to be certain that only significant cases are recorded, only those are included in which the left ventricle was markedly dilated (Fig. 2*A*). The incidence of this change is shown in Figure 1. It rises markedly with increasing body weight. In some of these cases considerable amounts of stainable fat were demonstrated in frozen sections; however, such sections are not available in a sufficient number of cases to warrant statistical evaluation.

Necrosis in the wall of arteries occurs in the heart and, less frequently, in the liver. This was described elsewhere,[6] and at that time the asphyxial nature of the lesion was postulated on a statistical basis, because of the frequent association with focal hemorrhages, and massive aspiration of amniotic sac contents. It was also found that the incidence increases with body size at birth; this corresponds well with the present data on the combined incidence of necrosis in the arteries of the heart and liver (Fig. 1). Necrosis occurs in the media at points corresponding to some but not all of those areas in which the adventitia is the seat of edema or hemorrhage (Fig. 2*B*). This, together with the fact that the lesion appears to progress from the outer border of the media inward, suggests that nutrition of the vessel wall derived from the adventitia is interfered with. The occurrence of these changes in coronary arteries has recently been confirmed by Ahvenainen and Landtman[7]; these authors have also connected them with asphyxia at birth.

Lungs.—The lungs play an important role in the perinatal distress syndrome, and this has been the subject of numerous investigations. Many of the changes which were encountered are well-known and need not be illustrated by photographs. However, their occurrence and significance merit consideration.

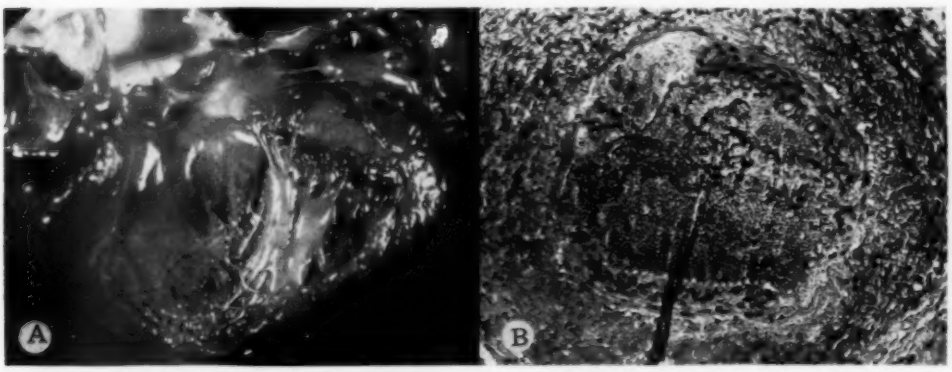

Fig. 2.—Cardiac lesions in perinatal distress. *A* (left), dilated left ventricle; *B* (right), focal necrosis in wall of coronary artery near area of hemorrhage in adventitia.

For the purpose of the present discussion, it is desirable to separate the pulmonary changes occurring before birth from those which develop when air breathing has taken place. It is important to remember that no direct information exists on the normal state of expansion of the lungs in utero. Stillborn infants may be assumed to have gone through a period of distress, and it is generally agreed that under these conditions abnormally deep respiratory movements may have taken place; the condition of the lungs as seen at autopsy is, therefore, no indication of the normal state in utero.

The lungs of very small, presumably nonviable fetuses have an immature gland-like

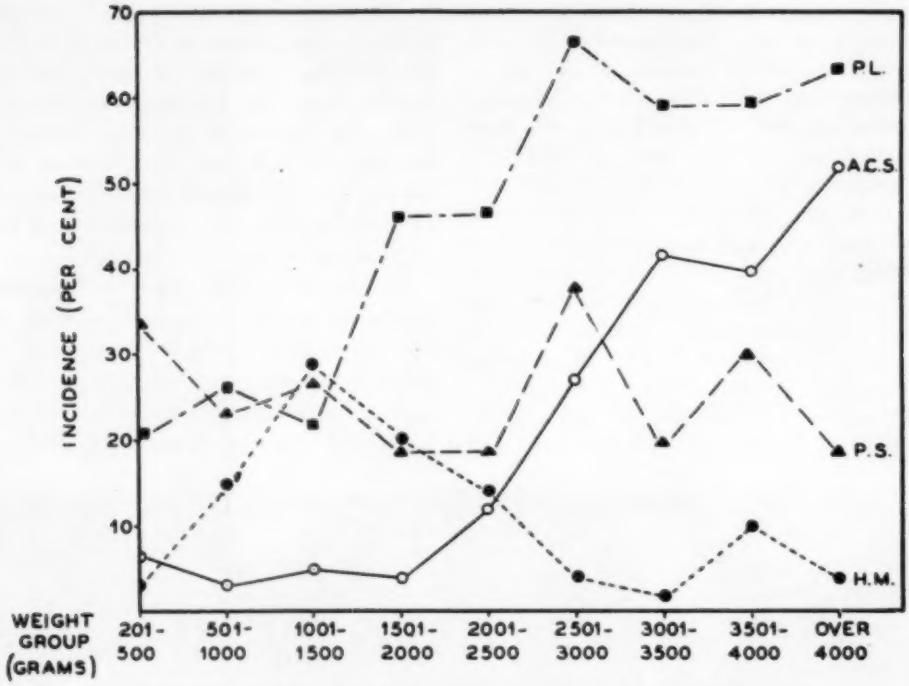

Fig. 3.—Incidence of pulmonary lesions by weight groups. A. C. S., aspirated solid amniotic sac contents; *H. M.*, hyaline membranes; *P. L.*, pneumonia in liveborn infants; *P. S.*, pneumonia in stillborn infants.

appearance. The lumina are lined by a cuboidal epithelium resting on a thick layer of connective tissue. At autopsy these spaces are filled with fluid, which in some instances contains considerable numbers of polymorphonuclear leucocytes. It is not certain that the presence of these cells indicates pneumonia, but they are counted as such in the distribution curve in Figure 3. A few of the spaces in these immature lungs may be aerated, and their walls will then be stretched and will assume an appearance resembling that of more mature lungs. In these early stages of development the appearance of the tissue gives no indication of the degree of distress present before birth. The degree of distension of fluid-filled lumina does not vary appreciably, and the amount of vernix caseosa present in the amniotic sac is insufficient for massive aspiration, which might be discovered on microscopic examination.

In the youngest viable infants, the alveolar walls are at first thicker than in mature lungs, but expansion and function are now possible. At the same time, a greater variation in the width of the lumen of fluid-filled alveoli is noted. It is not known to what extent the fetus aspirates amniotic fluid under normal conditions. In a previous report on this subject [8] various opinions were reviewed, and the conclusion was reached that large-scale breathing of fluid does not normally occur. If such aspiration did occur, one would expect large amounts of vernix to be aspirated into the lungs of every fetus; yet, it is generally agreed that the presence of much aspirated vernix is an indication of fetal distress. The microscopic appearance of this condition is well known. One finds large amounts of fluid containing desquamated cornified cells and amorphous fatty material in the bronchioles and alveoli. Pneumonia, as indicated by the presence of polymorphonuclear leucocytes in the alveoli, occurs in some cases with aspirated vernix, but occasionally also without the latter.

Aspiration of vernix apparently favors the development of pneumonia before or after birth but does not necessarily lead to it. Bacteria may or may not be found in sections showing leucocytic infiltration in stillborn infants.

The interpretation of the microscopic appearance of the lungs of liveborn infants dying within 3 days is not so obvious. Various new factors are introduced with the beginning of extrauterine life. Air enters the lungs and expands some of the bronchioles and alveoli to the limit of their capacity. It has been pointed out [8] that surface tension is a force counteracting the entrance of air into the alveoli; the same factor makes air accumulate in the largest possible—round—bubbles; this provides the smallest surface for a given amount of air. In contrast, the entrance of fluid affects all alveoli uniformly and never to the limit of expansion. Aeration of the lungs thus proceeds by an increase in the number of expanded alveoli rather than in the degree of expansion of each alveolus.[9] At the same time, fluid present in the alveoli at birth may be absorbed from the air spaces, and, on the other hand, edema fluid may leak into them just as edema may appear in other parts of the body. I know of no study in which amniotic fluid and edema fluid have been differentiated. Until this can be done, there will be no satisfactory interpretation of findings of fluid in the alveoli of liveborn infants; to a minor extent this problem may exist in stillborn infants as well. However, one may assume that pulmonary edema is of frequent occurrence in newborn infants in distress, because fluid-filled alveoli are a common finding. Considering the rapidity with which fluid is absorbed in the lungs, it is unreasonable to assume that fluid found at autopsy in the alveoli of infants who survived for several hours is amniotic in origin.

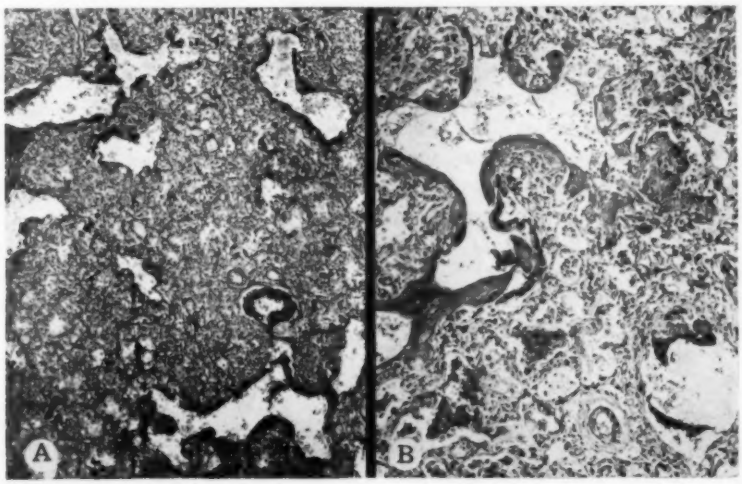

Fig. 4.—Hyaline membranes in the lungs of (*A*) a premature, and (*B*) a full-term infant. In the premature infant the alveoli are collapsed; there are no aspirated cornified cells. In the mature infant the alveoli are partially expanded with fluid and debris; the hyaline material contains cornified cells.

Another material present in the lungs of some newborn infants has also not been satisfactorily investigated, namely, that forming hyaline membranes. It appears microscopically as a homogeneous, apparently viscid layer lining some of the air spaces. The substance is found in this form only after air breathing has taken place, and it has been assumed that it is flattened out against the walls of air spaces by the entering air. In premature infants this condition characteristically prevails in the bronchioles and alveo-

lar ducts, and most of the alveoli are collapsed (Fig. 4*A*). A recent review of the subject [10] shows that fatty material may or may not be present in this hyaline layer; our own observation agrees with this. In mature infants the distribution of hyaline membranes differs from that in premature infants. The membranes are present in bronchioles as well as in some alveoli, and most of the alveoli are not collapsed, but partially expanded with fluid (Fig. 4*B*). Some alveoli may be aerated. It is known that hyaline membranes do not all consist of the same material, as shown by the wide variety of circumstances under which they occur.[11] In newborn infants the hyaline membranes of those born mature differ from those of prematures, in that they contain much more fatty material derived from vernix caseosa and numerous cornified cells from the same source. Figure 3 shows that the peak of incidence of hyaline membranes is near the lower border of viability, namely, in the weight group of from 1000 to 1500 gm. The nature of the materials which form the membranes in premature infants remains to be elucidated.

Hyaline membranes in the lungs of premature infants constitute an important problem because many instances of presumably fatal atelectasis are associated with, and perhaps caused by, these membranes in the bronchioles. Various theories of the nature, development, and significance of the membranes in the lungs of premature infants have recently been reviewed * and will not be discussed here. In judging the significance of hyaline membranes, one should remember that there is no proof that these membranes are the cause of atelectasis, since the same type of atelectasis characteristic of premature infants may occur in the absence of membranes as well.† Whereas true vernix membranes, as they occur at or near term, are obviously related to perinatal distress, this relationship is not so obvious in the case of what might properly be called "atelectasis of prematures frequently associated with hyaline membranes." It is not true, as has been claimed, that this condition occurs characteristically in babies who did well immediately after birth.[12] Reutter [14] has stated that hyaline membranes of premature infants occur after anoxia at or shortly after birth and then form part of a vicious circle which may lead to a fatal outcome. Landau [15] suggested that shock due to occult blood loss from the placenta may cause the formation of hyaline membranes. This part of the problem needs further elucidation.

The incidence of pneumonia in liveborn infants up to 3 days of age, as judged by the presence of polymorphonuclear leucocytes in the terminal air spaces, rises significantly about the time maturity is reached. No similar trend was evident in stillborn infants (Fig. 3). There is a striking similarity in the trend of the curve showing incidence of aspirated solid amniotic sac contents and that of pneumonia in liveborn infants. This suggests that aspirated vernix is a predisposing factor in pneumonia developing after birth, but not so much in intrauterine pneumonia. The latter may be due to intrauterine infection, which is not predicated upon any particular degree of maturity, or contents of the lungs.

Liver.—The liver is known to be susceptible to damage by anoxia. Three types of changes in the liver of newborn infants may tentatively be attributed to this cause. One of these is congestion of the capillaries; the second, degeneration of liver cells, and the third, necrosis of the walls of arteries.

Congestion is part of the generalized engorgement of vessels found in many of these infants. Microscopically one sees dilatation of sinusoids and, in severe cases, a reduction in thickness of the liver cell plates.

Degeneration of liver cells may manifest itself as cloudy swelling. Quite frequently the cytoplasm is very pale and contains few coarse granules; this is usually associated with appearance of a very distinct cell border, which earned these cells the designation "plant-like." [16] In other instances fat-free vacuoles appear in the cytoplasm. Fat-containing vacuoles are also seen. They may

* References 10 and 11.

† References 9, 10, and 12.

or may not appear in the liver cells in certain parts of the lobules, e. g., near the central veins. Reduction in the size of liver cells, resembling the change in chronic venous congestion in adults, is occasionally seen. Here, as in adults, it is likely that this is due, at least in part, to atrophy rather than pressure.[17] Finally, as the severest degree of cellular damage, focal necrosis occurs.

In some newborn infants a difference in the gross and microscopic appearance of the tissue in the right and left half of the liver exists owing to severer degeneration in the right half. In a previous report [17] 44 cases of this lateral difference were described, and additional cases have since been found. In that report the association with other signs of anoxia was pointed out, and it was concluded from these observations and from perfusion experiments that in the fetus only the left half of the liver receives, in addition to the usual supply, oxygenated blood from the umbilical vein, which protects it to some extent from the effects of anoxia. Severer degeneration in the right half may therefore be interpreted as a sign of intrauterine anoxia, even if it is seen in an infant who lived for a few hours or days after birth. Emery and Finch [18] have determined the fat content of the right and left lobe of the liver of newborn infants by extraction in vitro and have found a higher content in the right lobe in many instances. Emery [19] has described degeneration limited to the left lobe of the liver occurring as a normal process some time after birth. This is independent of the change described here.

The difference between the right and left lobe of the liver as found at birth is also significant from another point of view. It has often been argued, not particularly with reference to newborn infants, that some of the above-mentioned cytoplasmic changes are artifacts, or otherwise insignificant. Yet, if cloudy swelling or fat-free vacuolation of the cytoplasm occur more severely in one

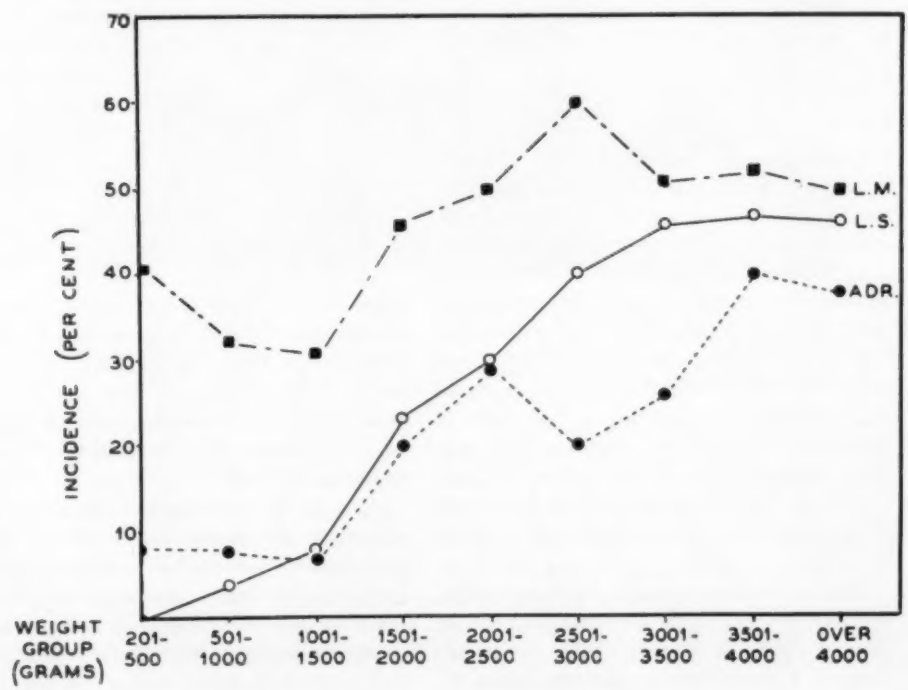

Fig. 5.—Incidence of lesions in the adrenal cortex (*ADR*) and of the liver (mild, *L. M.*; severe, *L. S.*) by weight groups.

half of the liver, this cannot very well be due to terminal or postmortem changes; it must have some real significance as an indication of changes occurring during life, if not as a sign of a functionally important disturbance. Atrophy and shrinkage of liver cells may similarly be unilateral; this militates against the assumption that venous pressure alone is the cause.

The various types of cytoplasmic degeneration may or may not be severer in the right half of the liver than in the left. In the scores which were prepared for the statistical evaluation of the present material, the intensity of these changes was given values ranging from 1 to 3, regardless of a lateral difference. In Figure 5 the occurrence of low degrees (valued as 1) is shown separate from that of severer forms (valued as 2 or 3). It is apparent that mild changes do not vary significantly with body size. Severe degeneration, on the other hand, was not found in the smaller infants and becomes more frequent as the birth weight increases. This indicates that not only the incidence but also the severity of lesions increases with body weight at birth.

Necrosis in the wall of arteries resembles that found in the heart.

Pancreas.—Involvement of the pancreas in neonatal asphyxia has been suspected on the basis of the occurrence of lesions in infants with severe anoxial lesions elsewhere in the body. Pancreatic lesions were included in the scores to be discussed later, but their incidence is not sufficiently high to warrant further statistical analysis. A total of 10 cases was found among 383 in which the pancreas was examined. In addition, a few cases of pancreatic lesions were available from material gathered prior to the present project; a striking association with other manifestations of the distress syndrome was found in these cases as well.

The lesions encountered are hemorrhage, necrosis, or leucocytic infiltration. None of these occurred so frequently that a description of a characteristic pattern might be given. It is necessary to distinguish this change from dense, focal infiltration by lymphocytes, which is a frequent occurrence in newborn infants and has no known significance.

Adrenals.—The adrenals showed a variety of changes which can all be traced to two basic forms, namely, congestion of blood vessels and focal necrosis. Congestion is a common occurrence in asphyxiated infants, and its severity may be out of proportion to that in other organs. The central portion of the adrenal is particularly affected in many instances. The adrenals may then appear enlarged, and their inner portions, dark red. This often leads to a diagnosis of adrenal hemorrhage. Microscopically the central vein is greatly engorged with blood, as are the capillaries in the central part of the cortex. However, a diagnosis of hemorrhage should not be made as long as the blood is contained in capillaries, no matter how severely compressed the cortical cell cords may be. This condition may extend peripherally to various degrees and may reach the capsule in small or large areas. If that happens, the adrenal appears hemorrhagic on the surface (Fig. 6*A*). However, the architecture may be preserved even in these cases, and cortical cell cords are then found between the congested capillaries (Fig. 6*B*). The cortical cells may be very thin and show other changes suggestive of degeneration; no attempt has been made to estimate the degree of their viability or functional capacity.

In other instances, or in some parts of these same adrenals, the parenchyma may completely disappear in areas of various sizes and give way to a pool of blood; a diagnosis of hemorrhage is then justified. The transition from areas of congestion to those of hemorrhage may be gradual (Fig. 6*C*) or abrupt (Fig. 6*D*).

Adrenal hemorrhage has often been considered as the result of mechanical trauma, particularly when the infant's trunk was subjected to bending or twisting such as may occur in breech delivery or with some obsolete methods of resuscitation. Goldzieher and Gordon [20] recognized a predisposition to traumatic hemorrhage in cases of hyperemia; the same authors found that extensive

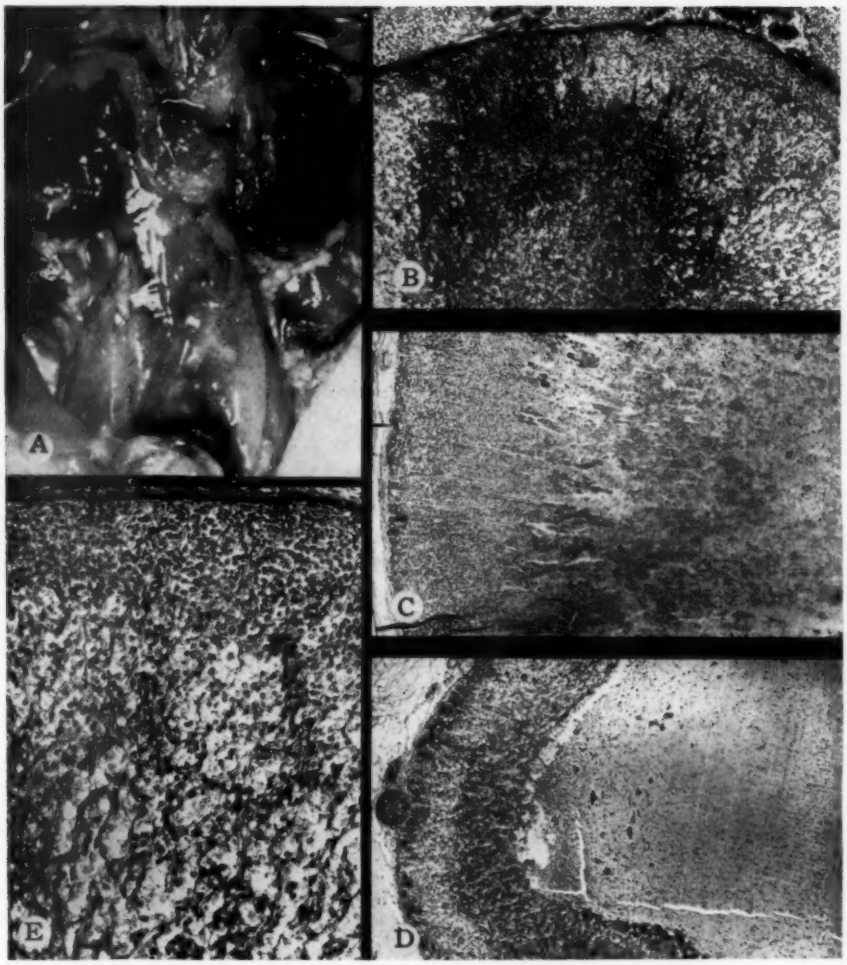

Fig. 6.—Lesions in the adrenal cortex referable to perinatal distress: *A* (left, top), bilateral hemorrhage in part of the adrenals. *B* (right, top), section showing area of extreme engorgement of capillaries with transition into hemorrhage; elsewhere there is focal necrosis. *C* (right, middle), congestion and hemorrhage in central portion. *D* (right, bottom), sharply demarcated hemorrhage in central portion of adrenal. *E* (left, bottom), focal necrosis of cortical cells.

hemorrhage is usually bilateral. Tow [21] stated that "there is no mechanical factor, but asphyxia is a fairly constant occurrence," and Macgregor [22] included adrenal hemorrhage among lesions caused by asphyxia. The present observations agree with the latter opinion. In none of the cases studied has there been a laceration of the adrenals and, as has been mentioned, the lesion is severest in the center. According to the criteria just described there is in many instances, including the one shown in Figure 6*A*, no hemorrhage at all but rather severe congestion possibly associated with degenerative changes in the parenchyma. The remaining cases in which there is true hemorrhage often show a transition from congestion or definite signs of degeneration of the parenchyma, which will subsequently be described. For these reasons it is believed that adrenal hemorrhage due to mechanical birth trauma is very rare, if it occurs at all.

Focal necrosis of cortical tissue has been found repeatedly; examples are shown in

Figure 6*B* and 6*E*. In its evaluation it is necessary to keep in mind that necrosis normally occurs shortly after birth in the central portion of the cortex, the so-called provisional or fetal zone. This normal involution occurs in a part of the cortex which has very large cells; it usually does not begin until some time after birth. On the other hand, necrosis as shown in Figure 6*E* occurs among the smaller cells in scattered areas of the permanent cortex and is often separated from the fetal cortex by intact tissue. Only in a few cases was there cause for doubt as to the nature of areas of necrosis. The cells of the affected areas appear disintegrated. The nucleus is not seen, and the cytoplasm is replaced by clear spaces or granular material. If several adjacent cells are affected, there may be a larger space filled with debris. In many of these areas there is no extravasation of blood; in other areas hemorrhage occurs; in extensive hemorrhage the recognition of previous necrosis is impossible.

One may suspect that disintegration of cortical cells is a postmortem change. No proof in the strict sense can be brought forward that this is not so. However, the following evidence suggests that the changes are significant: 1. They closely resemble those obtained in animal experiments (see below). 2. They are focal in distribution. 3. They may be associated with hemorrhage. 4. There is evidence that delay in preserving the tissue does not produce a higher incidence of lesions; the incidence of lesions valued here as 2 or 3 is not significantly different in babies who died during daytime, when bodies are promptly removed to refrigeration, as compared with deaths during the night, when delays in transfer are likely to be longer. There is also no significant difference between autopsies performed within one day after death and those done later.

No characteristic lesions have been found in the medulla of the adrenals, which is very poorly developed at birth. Even if centrally located areas of hemorrhage could damage the medulla, no functional disturbance should be expected, since other cells with the same function are scattered elsewhere in the body.

The incidence of lesions in the adrenals (Fig. 5) rises with increasing birth weight much in the same manner as do most other lesions described here. There is a high incidence in infants with other severe manifestations of distress. If the incidence of mild and severe lesions were plotted separately, the curves would resemble those for liver lesions; the severe changes increase more with birth weight than do the mild ones.

TABLE 2.—*Number of Cases in the Weight Groups of 2501 to 4000 Gm., Showing Various Conditions of the Lymphoid Tissue*

	Thymus			Spleen (Follicles)		
Amount of Lymphoid Tissue	Small	Medium	Large	Small	Medium	Large
Infants with scores of 20 or lower.....	0	4	1	2	6	6
Infants with scores of 51 or higher.....	3	5	1	12	8	1

At this point mention should be made of changes which, according to Redaelli and his group,‡ occur in the lymphoid tissues of newborn infants in response to hyperactivity of the adrenal cortex. In line with the well-known fact that adrenocortical hormone causes depletion of lymphoid tissue, these investigators found an exaggerated involution of thymus and splenic follicles in infants born in distress. Studies of lymphoid tissues were originally not included in the present work. At the suggestion of the publications just mentioned, the following test was made in order to determine whether or not examination of lymphoid organs should be included in future studies of neonatal distress. Among the weight groups from 2501 to 4000 gm., all cases with scores of 20 or below, and of 51 or above, were selected in an effort to contrast cases with mild and

‡ References 23 and 24.

severe distress. All available sections of thymus and spleen were graded arbitrarily according to the amount of lymphoid tissue seen microscopically. In some cases only one of these organs was available. The weight of the organs had not been determined with sufficient accuracy to be evaluated. The results are shown in Table 2. They indicate that the microscopic appearance of the spleen reflects depletion of lymphoid tissues in stress better than that of the thymus. This should not be interpreted as a definite result but rather as a suggestion that examination of lymphoid organs may be worth while in the study of neonatal distress.

Urogenital Tract.—In the organs of the urogenital tract no highly significant observations have been made. Congestion and focal hemorrhage appear frequently in the renal cortex and the gonads, and less frequently in the region of the prostate and seminal vesicles or in the uterus.

Jonsson [25] has described a form of renal failure in infants who went through a period of severe anoxia, which is similar to that known to follow shock from other causes. In his three cases the highest NPN values varied from 90 to 127; two recovered, and the third showed at autopsy only casts in the tubules. Renal lesions due to vascular changes in shock, such as described by Oliver and co-workers,[26] do not appear within the short period of time after onset of disturbances to which the present investigation is limited.

In the testes hemorrhage into the stroma at birth has been considered as normal by some investigators [27]; in the present material it appeared to be associated with other signs of asphyxia. Not so well-known is the occurrence of similar, though less severe, changes in the ovary.

The association of severe congestion and hemorrhage in the internal genitals with asphyxia is a tentative one. The rise in the

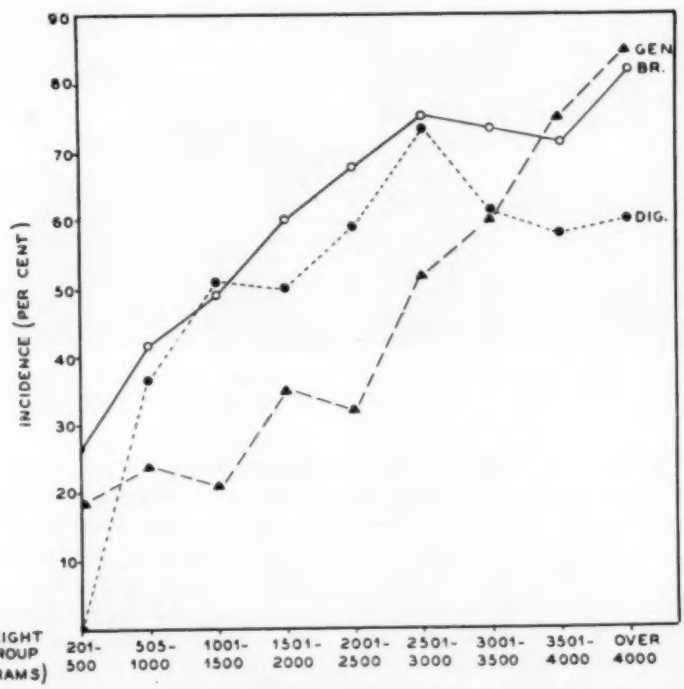

Fig. 7.—Incidence of lesions described in the text, by weight groups, in the brain, *BR.*; digestive tract, *DIG.*, and internal genital organs, *GEN.*

incidence of the lesions with increasing birth weight is very conspicuous (Fig. 7). What cannot be demonstrated by the present statistical data is the occurrence of severe hemorrhage, particularly in asphyxiated infants; yet, examination of individual cases has suggested this in many instances.

Gastrointestinal Tract.—Lesions in the gastrointestinal tract are associated with signs of asphyxia in a large number of infants, particularly those who survive for several hours after birth. Their interpretation was possible only on the basis of the assumption that asphyxiated infants are in shock and have so-called shock lesions.

The commonest and most significant lesions are those in the esophagus; they were previously described,[28] and no new information has since come to light. The mildest change, occasionally seen in stillborn infants, includes severe congestion of the propria, with mild hemorrhage and leucocytic infiltration. Severer forms, seldom found in infants surviving less than eight hours, include a marked infiltration of the mucosa by polymorphonuclear leucocytes and ulceration of varying extent and depth (Fig. 8*A*). As a rule, the severest change occurs in the lower portion of the esophagus, just above the cardia. For further details and illustrations the reader is referred to previous work.[28] Bacteria or fungi are demonstrable in the lesions in some of the cases and are presumably secondary invaders. Two cases of perforation are on record.[28]

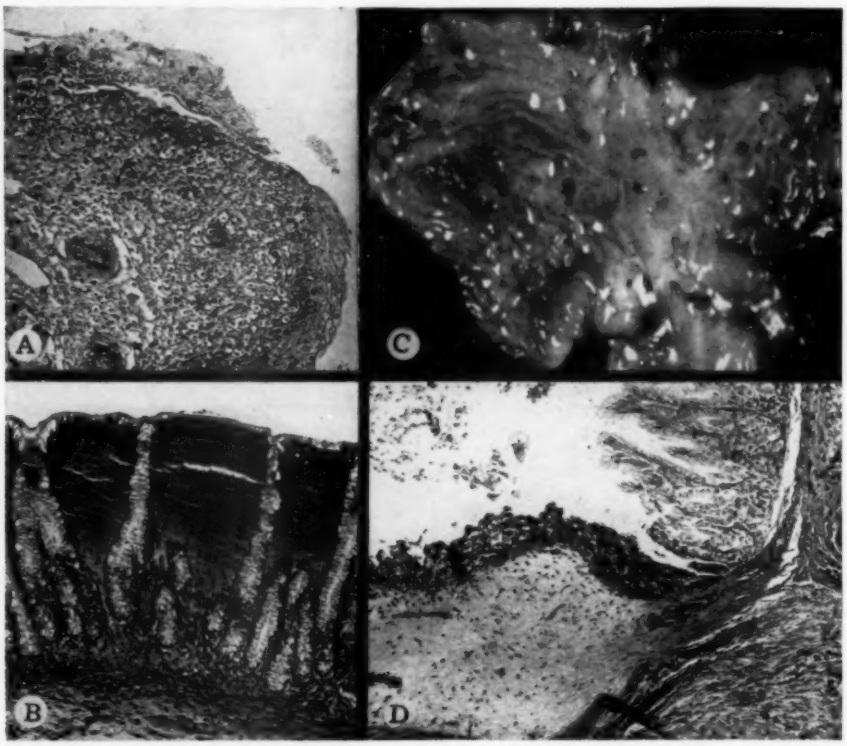

Fig. 8.—Lesions referable to distress in the esophagus and stomach: *A*, esophagitis; *B*, mucosal hemorrhage in the stomach; *C*, gross, and *D*, microscopic appearance of erosions in the stomach.

Another pathological change resembling those known to occur in shock[29] occurs in the stomach and duodenum. It consists of areas of various sizes in which the mucosa shows extreme congestion of blood vessels

with extravasation of much blood. The epithelium may be well preserved (Fig. 8*B*), or the entire mucosa may have the appearance of hemorrhagic necrosis.

A third type of lesion occurring only in the stomach is poorly understood. The impression was gained that it, too, occurs particularly in infants who had been in distress, and it is therefore tentatively included here. This lesion, which for want of a better term will be referred to as erosion, is grossly visible as a sharply outlined, translucent, gray, depressed area of the gastric mucosa (Fig. 8*C*). Most commonly there are multiple areas of this kind scattered in the stomach wall; occasionally only one or two are seen. Microscopically (Fig. 8*D*) the mucosa appears shrunken to the extent that its architecture can no longer be made out; however, the tissue contains well-stained nuclei and does not seem to be necrotic. The submucosa underlying these areas consists of swollen, homogeneous fibers. Serious consideration must be given to the possibility that all or part of these changes are artifacts and therefore of no pathological significance. The sharp demarcation of the lesions and their association with other changes described here speak, in my opinion, for the assumption that they are not entirely artificial but, perhaps, minor pathological changes aggravated post mortem by the action of gastric juice. It seems plausible that the swelling of the submucosa in particular is an artifact due to the action of an acid medium upon the connective tissue in an area in which the covering mucosa was damaged in vivo.

For the purpose of statistical evaluation, all the above-mentioned lesions of the digestive canal, of which esophagitis is the commonest, have been combined; their occurrence in the usual weight groups is shown in Figure 7. It is apparent that the incidence rises until it reaches approximately 60% at about the stage which is customarily considered as the border of prematurity (2500 gm.).

The lesions of the digestive tract described here may be significant because of hemorrhage, secondary infection, or, rarely, perforation.

Cerebral Lesions.—Several kinds of cerebral lesions in newborn infants are well known. There are instances of obvious mechanical trauma, particularly those associated with tears of the falx or tentorium and subdural hemorrhage. On the other hand, it is generally accepted that scattered focal hemorrhages in the brain tissue are due to anoxia; the same cause is invoked in instances in which later in infancy rarefaction of brain tissue is found, presumably as a sequel of asphyxial necrosis.

In addition to these clear-cut instances, there is a wide variety of others in which the share of trauma and anoxia is not so obvious. Cases in which mechanical damage to vital cerebral centers (often by pressure of a hematoma) causes anoxia need no further discussion at this point. More problematic are those in which anoxia predisposes to, or simulates, mechanical lesions, e. g., intraventricular hemorrhage of premature infants, which was recently studied in the present material.[30]

Evaluation of a series of 50 cases of subependymal cerebral hemorrhage, with or without perforation into the lateral ventricle, led to the following conclusions: Subependymal hemorrhages vary in size and number and usually originate from small vessels. They are not due to mechanical trauma, but their severity and the probability of perforation into the ventricle are increased in cases of cephalic presentation, presumably by a difference between the presenting head and the rest of the body enclosed in the contracting uterus (*Ansaugungsblutungen* of Schwartz[31]). Asphyxia with its concomitant venous congestion and vascular damage is believed to be the primary cause. This has also been the opinion of Morison[32] and Macgregor.[22] The immature subependymal tissue of premature infants with its poorly supported blood vessels is particularly vulnerable and is, therefore, much more frequently and severely affected than are other parts of the brain. Gröntoft[33] has shown

evidence that venous congestion rather than increased permeability of vessels is the cause of subependymal hemorrhage.

Other types of intracranial hemorrhage are not as readily analyzed as was the example just given. Yet it is obvious that the role of asphyxia in intracranial hemorrhage is more significant than is usually appreciated. Many instances of subarachnoid hemorrhage are sequelae of asphyxia rather than mechanical trauma.§ This is readily seen in those cases in which foci are scattered over the surface of the brain. More frequently the areas of hemorrhage spread and coalesce, and then an evaluation of their distribution and of other associated changes is necessary in order to determine the most probable cause.

Cerebral changes were statistically analyzed in a manner similar to that of other lesions described above (Fig. 7). While the usual trend toward a more frequent occurrence in large infants is well marked, there is a fairly high incidence in premature infants, reaching nearly 50% in what is often considered to be the youngest viable group (1001 to 1500 gm.). This reflects not only the frequent occurrence of subarachnoid hemorrhage of moderate degree, but particularly the large number of cases with subependymal foci.

COMMENT

1. Relationships of Various Pathologic Lesions to the Effects of Distress

Some of the lesions described in the preceding sections are known effects of anoxia, such as, for instance, petechiae in the thoracic viscera or massive aspiration of vernix caseosa into the lungs. These require no further discussion. Other findings will now be considered with a view to their possible relationship to shock or the acute phase of the adaptation syndrome.

Edema or, in severe cases, hydrops appears to be a sign of distress.‖ Its relative frequency and severity at birth, as compared with older-age groups, has been explained [24] by the unlimited supply of fluid which the fetus may receive from the placenta. Whenever capillary damage leads to extravasation of fluids, this is not limited to the amount available at one time from the blood plasma through hemoconcentration, but, rather, unlimited because the blood volume is constantly replenished in the placenta. For this reason, shock in the fetus should produce hydrops rather than hemoconcentration with moderate edema. This has been confirmed by the experimental production of hydrops after intraperitoneal administration of muscle pulp to guinea pig fetuses in utero.[34] Edema and hydrops are therefore produced by capillary damage, including shock in the fetus, and are thus signs of fetal distress. It is known that the occurrence of hydrops is not limited to isoimmunization.¶ Of particular interest is the possibility that many newborn infants in distress have pulmonary edema. The occurrence of this condition in shock has been emphasized by Moon,[2] and the same author states that edematous lungs are particularly liable to secondary infection and pneumonia. Pulmonary edema, atelectasis, and aspiration of vernix caseosa, all found in infants born in distress, are important predisposing factors, even though none of them will invariably cause pneumonia. Aspirated vernix and edema are probably responsible for the higher incidence of pneumonia in the larger infants, in whom they occur more frequently than in the small ones. A competent discussion of pneumonia in newborn infants has been presented by Macgregor,[37] who has recently used the term "asphyxial pneumonia." [22]

Necrosis in the wall of coronary and hepatic arteries was recognized as part of the perinatal distress syndrome by its occurrence in association with other lesions of that syndrome.[6] As was mentioned above, tissue damage in the adventitia is probably a significant factor in the pathogenesis of this lesion. A few reports of suggestively

§ References 22 and 23.

‖ References 22 and 34.

¶ References 35 and 36.

similar lesions in various parts of the body were cited in a previous article.[9] In addition, a recent observation of Waters [38] is of interest: Segmental necrosis in coronary and other arteries similar to that occurring in newborn infants was found in dogs after their blood pressure had been raised for short periods of time by the administration of pressor substances. So far, this observation has not led to progress in the interpretation of arterial necrosis of newborn infants, except that one might suspect that temporary vasoconstriction, which is known to play a role in the development of shock lesions,[29] may be a common factor in both instances.

In the liver, nonspecific cytoplasmic degeneration occurs in anoxia as well as in shock.[16] Dorkin and Weinberg [39] have claimed that accumulation of histologically demonstrable fat in the newborn infant's liver is a normal process; Benitez,[40] on the other hand, has related this process to anoxia. A gross difference between the right and left half of the organ in the fat content of liver cells suggests that anoxia is the cause,[17] perhaps superimposed upon whatever storage of fat may normally occur. If a difference in fat content between the halves of the liver were due to absorption of fatty substances brought in from the placenta, the larger amount should appear in the left half, which receives blood direct from the umbilical vein; actually, the reverse has been true in all instances. The occurrence of cytoplasmic degeneration of the liver cells in those infants who have other signs of distress further suggests that the changes in the liver are related to the same cause. Emery [19] has described atrophy in the parenchyma of the left lobe of the liver following birth, related to cessation of the flow of umbilical vein blood through that tissue. This mechanism, if substantiated, is a normal occurrence and unrelated to the one discussed here.

Lesions in the pancreas, as described here, are conspicuous by their association with pathologic signs of severe distress. This may be understandable in the light of Selye's [41] observation that in the alarm reaction "acute involution" of the pancreas occurs, which may lead to necrosis. According to the same source, "It is possible that the acute pancreatitis occasionally seen in man after burns and other types of sudden stress is related to this phenomenon." With this in mind, the lesions encountered here have tentatively been classified as part of the perinatal distress syndrome.

It is well known that the adrenals react prominently in the course of the alarm reaction which includes shock. Selye,# has suggested that adrenal hemorrhage occurring in the Waterhouse-Friderichsen syndrome, which is similar to that described here, is the result of a severe reaction incited by fulminating infection. According to Kloos and Staemmler,[44] "apoplexy" or involution of the adrenal cortex may be due to an excessive demand during and after birth, comparable to the alarm phase of the adaptation syndrome. Mosca and Corbetta [24] have examined the adrenal cortex in newborn infants with a view to demonstrating the effects of the alarm reaction at birth. They have found excessive loss of lipid substances in infants who were in distress at birth. Venning, Randall, and György [45] have found that the adrenal cortex of the newborn infant is capable of discharging increased amounts of glucocorticoid in response to trauma (in a wide sense). These considerations justify the hypothesis that the lesions of the adrenal cortex described here are reactions to stimulation in shock. There is a close resemblance to changes produced in experimental animals by hormonal overstimulation of the adrenal cortex.[46] It would be desirable to confirm the existence of adrenal damage by tests done on living infants after distress at birth.

The occurrence of swelling with hyperemia, edema, and hemorrhage in the internal genital organs and the mammary glands has been mentioned here because severe degrees of these hemorrhages are seen par-

References 42 and 43.

ticularly in infants with extensive distress lesions. On the other hand, it has been generally accepted that the hormonal environment of the fetus produces some of these changes.[47] It remains to determine to which extent each of these factors may affect the genitalia and breasts.

Lesions in the digestive canal, of which esophagitis is the most significant, are strikingly similar to those described as shock lesions in experimental animals and in human subjects.[29] As was previously pointed out,[28] no other satisfactory explanation of the high incidence of esophagitis in infants has been found. In order to give an indication of the frequent association of esophageal and gastric lesions with others characteristic of anoxia, reference may be made to a tabulation of the occurrence of various changes in a group of infants with degeneration of the liver predominant in the right half: in that group with a high incidence of petechial hemorrhage, massive aspiration of vernix caseosa, and necrosis in the wall of arteries, 12 of 16 liveborn infants had esophagitis or gastric erosions.[17]

Most of the lesions described here occur also in older children and adults under various circumstances. Yet one seldom finds them as numerous and severe in older subjects dying from asphyxia as in the newborn. There is no definitive explanation for this difference. The ability of the newborn infant to subsist to some extent on anaerobic metabolism,* which may be lifesaving in many instances, may well prolong life in other cases just long enough to allow severe lesions to develop. Adults with comparable degrees of anoxia probably succumb more rapidly and therefore show fewer visible changes.

The mutual relations of anoxia and shock, on the one hand, and mechanical birth injury, on the other, were previously discussed.[1] Both anoxia and shock may be caused by mechanical trauma, the former particularly by brain lesions. It is possible, though this cannot be objectively determined, that trauma sufficient to produce distress may occur without any grossly visible lesion such as, for instance, tears of the tentorium or rupture of the liver.

Anoxia may predispose organs to mechanical trauma by damage to the parenchyma and the capillary walls, as well as severe congestion of blood vessels. Disruption of tissues and hemorrhage of significant extent may thus occur in organs which would otherwise not have been greatly affected by the same trauma. The role of congestion in the production of certain types of intracranial hemorrhage has been demonstrated by Gröntoft.[33]

Finally, there are some lesions significant statistically because of their frequent occurrence, which are due to anoxia and distress and have been mistaken in the past for the effect of mechanical trauma because they are associated with hemorrhage. One of these, occurring in the adrenals, has been discussed here. The other one is intraventricular hemorrhage in the brain as it occurs in premature infants. This was frequently mentioned here and more extensively discussed in a previous report [30] and by Gröntoft.[33] It was demonstrated that even a very conservative consideration of asphyxia as a cause of intracranial hemorrhage changes the figures of the incidence of trauma to a great extent. When only those cases are withdrawn from the classification of trauma in which intraventricular hemorrhage could be demonstrated to arise from subependymal foci, figures for intracranial birth trauma in premature infants drop to a fraction of the previous values.[30] Actually, it is very likely that even those instances of intraventricular hemorrhage in which no subependymal focus could be detected as a source are also due to the same cause, so that the figures for mechanical trauma in premature infants should be further reduced. With proper evaluation of these factors, figures for mechanical injury at birth as a cause of death should be much lower than that given as 16.6% in a recent study.[50]

In recent years several investigators have suggested the existence of a state of shock

* References 48 and 49.

in newborn infants who are in poor condition. Miller [51] supported this by evidence of hemoconcentration during the first two postnatal days. Berlind [52] suggested that fetal shock might be treated at birth with hormones of the adrenal cortex, and Åkerrén [53] referred specifically to a state of shock preceding death in premature infants. Govan [54] distinguished on the basis of autopsy findings three groups of cases showing, respectively, asphyxia, shock, and a combination of the two; his observations took into account various patterns of congestion of blood vessels and focal hemorrhages. As was mentioned above, a strict separation of asphyxia in neonatal distress seems impractical and undesirable because of their close mutual relationship; however, the relative severity of these two factors may vary from case to case.

Redaelli and Cavallero [23] and Mosca and Corbetta [24] have given detailed consideration to the birth process of the infant as an alarming stimulus, in the sense of Selye, and have discussed endocrinologic aspects of the "diseases of adaptation of the newborn." Similar thoughts were expressed by Obes Polleri,[55] who referred to *crisis de nacimiento* of premature infants. Schäfer [56] referred to birth as a traumatic *Eingriff* affecting the infant's organism; he mentioned the existence of signs of shock and compared the condition with Selye's alarm reaction. As stressor agents, Schäfer considered trauma, anoxia, and cooling. Kloos and Staemmler [44] have studied the adrenals of newborn infants and have considered these infants to be in the alarm phase following birth. Ribadeau-Dumas and Heraux [57] reviewed numerous pathologic lesions of newborn infants, some of which they compared with those known to occur in shock. As mechanisms they considered, along with disturbances in the pituitary and adrenals, the effects of anoxia and of irritation of the central nervous system. Wickster† has called attention to a special cause of shock in the newborn infant, namely, blood loss from ruptured fetal vessels in the placenta or umbilical cord, and very recently this has been emphasized by several investigators.‡ Morison [63] holds that the comparison of the manifestations of neonatal distress with shock is of little value and that "it is premature to attempt to integrate them into the general adaptation syndrome of Selye." His contention is based, at least in part, on the assumption that the lesions in question are not common and "will only reveal a fraction of all cases of asphyxiation." In contrast, the data given in the present Figures 5 and 7 show that, e. g., changes in the adrenals and in the digestive tract which are those most strongly suggestive of shock occur in about 40% and 60%, respectively, of unselected autopsies on full-term infants.

2. Effect of the Degree of Maturity on Pathologic Changes Occurring in Distress at Birth

It was shown here in several graphs that almost all lesions of the distress syndrome occur more frequently in large infants than in small ones. The same trend is evident when the total scores of all infants are tabulated (Fig. 9). It should be kept in mind that this graph is based on arbitrarily assigned values; thus, for instance, necrosis in the coronary arteries may be valued as highly as esophagitis of medium severity. Yet, the fact that the results follow a consistent pattern speaks for the validity of these scores as a rough indication of the severity of the pathologic distress syndrome.

The increase in the severity of the distress syndrome with increasing body size (Fig. 9) may at first seem unexpected, because small premature infants are generally believed to be greatly affected by anoxia and also because their capillaries are known to be less resistant mechanically than those of older infants.[64] On the other hand, prematurity in itself is often a primary or contributory cause of death. Anoxia and distress recede in statistical value as a factor contributing to neonatal death to the extent that another factor, namely, prematurity, in-

† References 58 and 59.

‡ References 60 through 62.

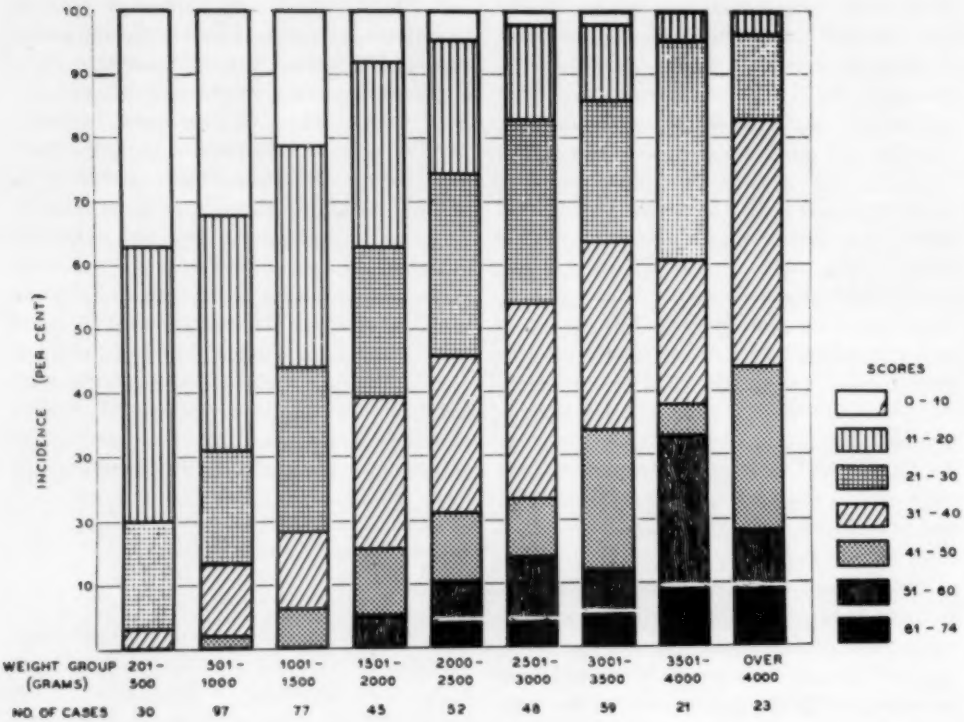

Fig. 9.—Scores of pathologic changes referable to perinatal distress, prepared as described in Table 1.

creases in significance in the lower weight groups. It is further possible that the young fetus lacks the ability of integrated reaction to injury which may be required for the production of the distress syndrome. This is suggested by the observations of Jailer and co-workers [65] that premature infants do not show an adequate response of their eosinophile count to epinephrine, whereas infants born at term do respond. According to these authors, the pituitary of premature infants is unable at birth to stimulate the adrenals sufficiently to produce the reaction. Another factor which may account in part for the present observation is that small infants usually go through a shorter and easier process of birth. The effect of the well-known greater resistance in the fetuses to anoxia § cannot be appraised at this time. It is possible, but not proved, that within certain limits premature infants are more effectively protected by this mechanism than are those born at term. Moreover, it has repeatedly been demonstrated, most recently by Walker,[66] that the oxygen reserve available to the fetus decreases during the latter part of pregnancy. This may bring the fetus near term more readily to the border line of tolerable anoxia than the premature one.

In view of these many factors which cannot be reliably evaluated, the cause of the lower incidence of asphyxial and related lesions in premature infants remains unknown. However, the fact remains that large newborn infants are more likely to have severe changes which may merit pediatric consideration. The data given in Figure 9 do not reflect the full increments in the extent of the pathologic condition. The severity of the lesions increased more with advancing

§ References 48 and 49.

birth weight than can be shown by these crude scores. Severe lesions are rare in small premature infants.

Two of the common pathologic lesions in premature infants are characteristic of that period and do not appear in mature infants in the same form; these are subependymal and intraventricular cerebral hemorrhage and a peculiar form of pulmonary atelectasis frequently associated with hyaline membranes. In the case of the cerebral lesions, we know the anatomical basis for its occurrence in premature infants only; it is the existence of a mass of undifferentiated subependymal tissue in certain parts of the walls of the lateral ventricles in which hemorrhage occurs with great ease. No satisfactory explanation is available for the pulmonary lesion. It has been pointed out that hyaline membranes themselves are not characteristic of newborn premature infants because they occur under many other circumstances.[11] The characteristic change is a peculiar form of atelectasis that is frequently associated with hyaline membranes but also occurs without these.[10] It is therefore believed that investigation of the hyaline membranes themselves will not solve this problem, but that a structural or functional peculiarity of the lungs of premature infants may well be responsible for this form of atelectasis.

3. Significance of Lesions of the Distress Syndrome in the Living Infant

Whatever the cause and duration of anoxia may be, an infant may appear in severe distress and acute danger at birth, sometimes without any suspicious signs during labor. These emergencies have been taken care of largely by introducing oxygen into the lungs as rapidly as possible. By relieving anoxia, this also eliminates the cause of shock, and in many instances the shock lesions will then disappear without further attention. Yet, the present findings suggest that in severe cases additional treatment may be directed, on the one hand, toward shock and, on the other hand, toward lesions in certain organs.

It remains for future clinical studies to tell whether or not an objective determination of the existence of shock in asphyxiated newborn infants is possible and practical. The usual criteria such as changes in body temperature, the quality of the pulse, blood pressure, and evidence of hemoconcentration are not reliable in newborn infants, and it may be necessary to test the usefulness of treatment for shock empirically. Since many of these infants have an excess of extravascular fluid, which may or may not be clinically evident as edema, and the erythrocyte count is usually high, the administration of whole blood should not as a rule be necessary. In order to draw fluid from the tissue spaces, it is suggested that a hypertonic colloid, such as concentrated plasma albumin, may prove useful. A gradual, rather than sudden, increase of the blood volume may be desirable in view of possible myocardial damage and the danger of heart failure.

Among lesions of vital organs which might merit therapeutic consideration are those of the heart, liver, and adrenal cortex. In contrast to what was just said about shock, laboratory methods may become available to test the functional significance of these organ lesions before definite suggestions for treatment are made.

Concerning the heart, pathologic evidence of damage and failure is convincing. In addition, severe congestion is frequently encountered in asphyxiated newborn infants. These changes may be aggravated by a reduced blood flow through poorly expanded lungs. The first electrocardiographic evidence which would corroborate these findings has recently been obtained by Ahvenainen and Landtman.[7] Cardiologists will have to determine whether or not drug treatment is in order. The necessity of avoiding an excessive burden on the heart of anoxic babies was just mentioned.

The significance of liver damage for the surviving anoxic infant is unknown. It would be of interest to acquire pertinent information by liver function tests in living infants. As in many other problems related to the newborn, the principal difficulty lies at this time in our lack of well-founded knowledge

of normal functional behavior of the newborn infant's organs and the normal values to be obtained by tests during the neonatal period. There are indications of functional immaturity in the newborn, and particularly in premature infants,‖ and it remains to be determined whether damage due to anoxia may be superimposed upon this. It is possible that liver damage may contribute in some instances to the so-called physiologic jaundice of the newborn infant.

Pathologic evidence of damage to the adrenal cortex in distress at birth has been brought forward here, as well as by other investigators mentioned above. Treatment of shock at birth with adrenal preparations has been suggested by Berlind.[52] This has, to the best of my knowledge, never been carried out in a systemic and well-controlled manner that would permit valid conclusions. Klein [68] has emphasized the need for conclusive studies in this field.

SUMMARY

A group of common pathologic lesions in stillborn infants is described. These changes are part of a nonspecific reaction to distress in utero or at birth, caused by anoxia, mechanical trauma, or other unfavorable influences of the environment. The appearance and occurrence of the component parts of this perinatal distress syndrome in 452 autopsies are described.

Apart from aspiration of amniotic sac contents, the lesions are related to three basic types of changes, namely, (1) circulatory disturbances (capillary damage, venous congestion), (2) parenchymal degeneration, and (3) secondary infection. Some of the changes are identical with those known to occur as "shock lesions." The central nervous system and most of the viscera may be affected.

Some of these pathologic changes suggest, as has been noted by other investigators on the basis of a variety of evidence, that newborn infants in distress are in shock.

The manifestations of the perinatal distress syndrome in premature infants differ qualitatively and quantitatively from those in mature ones. Lesions occurring in both groups are apt to be less severe and numerous in premature infants. On the other hand, there are common and highly significant changes characteristic of premature infants, notably intraventricular cerebral hemorrhage and a peculiar form of atelectasis frequently associated with hyaline membranes; the relationship of the latter to perinatal distress is not quite clear.

On the basis of the present findings it is suggested that pediatricians consider the possibility of treating newborn infants in distress for shock as well as for insufficiency of the heart, liver, and adrenal cortex, in addition to providing a supply of oxygen. Clinical observation and laboratory tests should be used to decide on the merits of this suggestion.

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Autoradiographic and Histopathological Studies of Thorium Dioxide Patients

The Deposition of Thorium and Its Daughter Radioelements in the Soft Tissues and Skeleton Following Thorium Dioxide Administration

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Considerable interest has recently been created about the utilization of radioactive thorium (Th^{232}) as a breeder material in the production of atomic energy. Information about the late effects of thorium dioxide (Thorotrast) will be helpful in protecting the personnel that may be exposed to thorium. In addition, this information may lead to a better understanding of the late clinical effects of other radioelements in medical, military, and industrial use today.

Thorotrast was first used in diagnostic radiology in this country in 1930. It contained 20% thorium dioxide by weight. The amount of thorium used for a hepatogram (75 cc.) has an estimated alpha ray equivalent of 1.4γ of radium and an estimated gamma ray equivalent of 1.5γ of radium, assuming that both radium and thorium are in equilibrium with their daughter products. The physical half-life of thorium is 1.39×10^{10} years.[13] The biological half-life of thorium dioxide has never been accurately determined; however, it seems that almost all of the injected material remains in the body indefinitely.*

There have been relatively few clinical changes reported following the administration of thorium dioxide.† Histopathological changes have been reported in the liver, spleen, bone marrow, bone, lymph nodes, adrenals, lungs, and kidneys.‡

Listed below are brief summaries of the case histories of two patients who died 10 and 19 years after thorium dioxide administration.

Patient A. D. died in 1953, age 85 years, from generalized arteriosclerosis. In 1934, 75 cc. of thorium dioxide was administered in an attempt to determine the etiology of an enlarged liver.

Patient S. L. was given 72 cc. of thorium dioxide as an aid in the diagnosis of echinococcus cyst of the liver in 1937. The patient died in 1947 from chronic leukemia.

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* References 4 and 12 and Hursh, J.; Stedman,

† References 4, 8, and 16 and Looney, W. B., published data.

† References 4, 8, and 16 and Looney, W. B.; and Colodzin, M.: Unpublished data.

‡ References 1, 3, 4, 10, 14, 15, and 17.

OBSERVATIONS

Aggregates of thorium dioxide with a marked variation in size were found throughout the liver. The largest aggregates, containing most of the thorium, were found in the peripheral areas (Figs. 1 and 2). In general, there was little reaction and destruction in Patient A. D., who had thorium dioxide for 19 years (Fig. 3). An exception to this was the spleen, which had contracted to about one-half its normal size (Fig. 4). Marked destruction and contraction with fibrous connective tissue replacement was present (Fig. 5). The autoradiographs of the spleen (Fig. 6) show the marked concentration of alpha tracks and fibrous connective tissue replacement of the normal splenic architecture. Increased thickness of the walls of the blood vessels was occasionally found (Fig. 7).

Small deposits of thorium dioxide were present in the medulla, and small particles were found in the inner aspects of the cortex of the adrenal of Patient S. L. (Fig. 8).

Very large deposits of thorium dioxide were present in the bone marrow of the vertebrae. Many of the deposits in the marrow of the long bones were adjacent to the endosteal surfaces (Figs. 9 and 11). Small particles were also found in the central canals of the Haversian systems (Fig. 10). Autoradiographs of the three serial sections of compact bone from the shaft of the femur were made, and the alpha tracks found in the emulsion were counted after a three-day exposure. A considerable percentage of the alpha activity observed in the emulsion appeared as four- or five-pronged stars (Fig. 12). The alpha activity in the skeleton was very small compared with the activity in the liver and spleen. Radiochemical analysis of the radium isotopes of the thorium decay series and spectrochemical analysis of thorium and its isotope radiothorium were made on bone specimens from these patients. Minute amounts of both thorium and the radium isotopes were found in various parts of compact and cancellous bone of patient A. D. 19 years after thorium dioxide administration.§

COMMENT

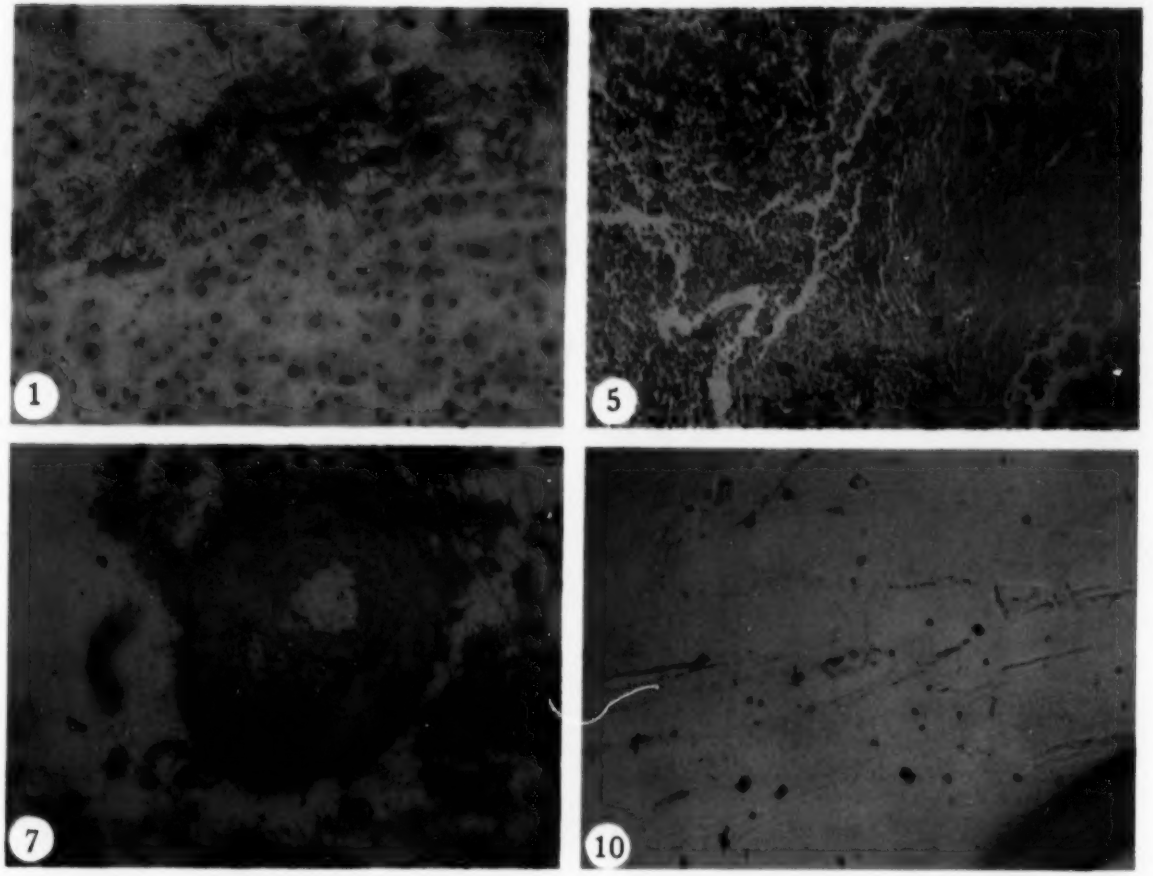
Almost all of the thorium given intravenously remains in the body indefinitely. Even though some minor migration may take place, sites of deposition seem to remain the same throughout the life of the patient.‖ Calculation of the radiation the body will receive from thorium dioxide administration is very difficult because of the large number of daughter radioelements in the thorium series. An understanding of the distribution and rate of elimination of the daughter products, as well as of thorium, is necessary for accurate determinations of the radiation dose.

Determination of total body gamma ray activity and spectrochemical and radiochemical analysis of tissues from the various organs are some of the techniques that have been used in the present investigations. Utilization of these techniques has given additional information, which will be helpful in the estimation of the ionizing radiation the body receives following thorium deposition. The short range of the alpha particles in relation to the large diameter of the thorium deposit may account for the comparative lack of irradiation damage seen in sections of the liver of the patient who had had thorium dioxide for 19 years. Hyaline changes in the walls of blood vessels and constriction and occlusion of the lymphatics have been reported.¶ This thickening and partial occlusion of the blood vessels most likely causes additional destruction and fibrosis to the organ distal to the affected blood supply. In the spleen there appears to be a progressive destructive process. The destruction of the

§ Hursh, J.; Stedman, L. T.; Colodzin, M., and Looney, W. B.: Unpublished data.

‖ References 4 and 11 and Hursh, J.; Stedman, L. T.; Colodzin, M., and Looney, W. B.: Unpublished data.

¶ References 7 and 11.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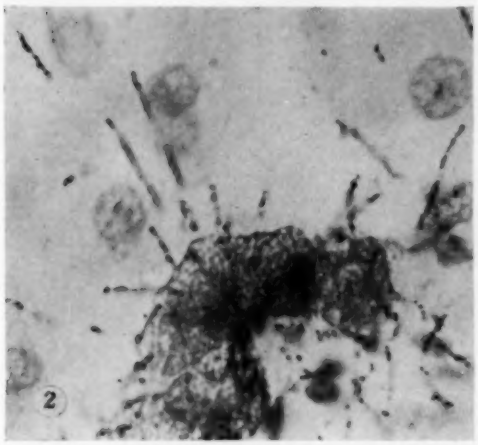
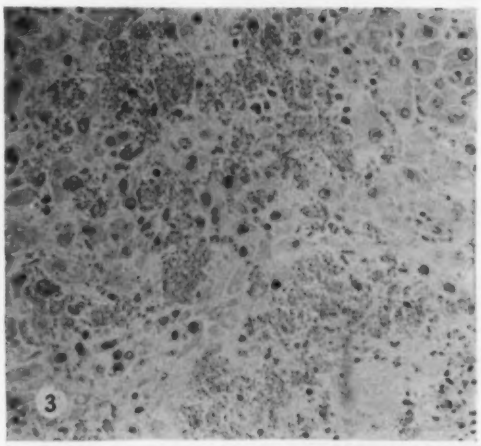




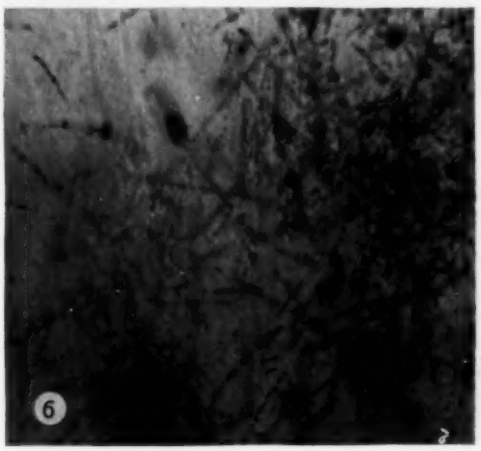


Fig. 1 (Patient S. L.).—Detailed autoradiograph of the liver. Note the large aggregate of thorium dioxide in the periportal area and the short range of the alpha tracks in relation to the large diameter of the aggregate. Smaller particles of thorium dioxide and alpha tracks are seen in the sinusoids of the liver lobule; reduced from mag. × about 450.

Fig. 2 (Patient S. L.).—Detailed autoradiograph showing a thorium dioxide aggregate, the alpha tracks, and the adjacent hepatic cells; reduced from mag. × about 950.

Fig. 3 (Patient A. D.).—This is a histological section of the liver, showing numerous aggregates of thorium dioxide in the liver, with little destruction and almost no fibrous connective tissue surrounding the aggregate; reduced from mag. × about 900.

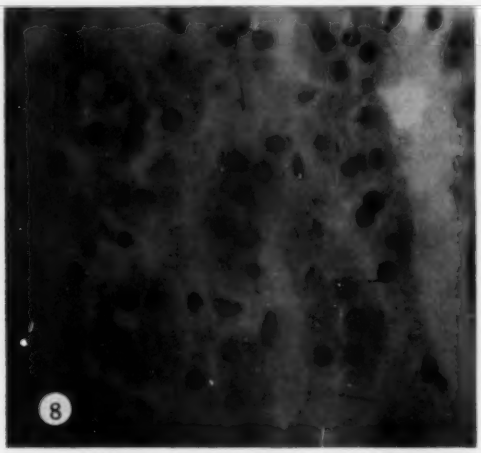
Fig. 4 (Patient A. D.).—Cross section of the spleen. As a result of the replacement of the splenic architecture with fibrous connective tissue, it has contracted to about one-half its normal size.

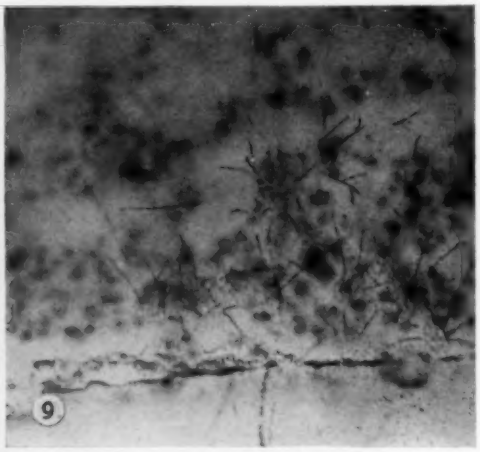
Fig. 5 (Patient A. D.).—This histological section of the spleen shows the marked deposition of thorium dioxide, destruction of splenic architecture, and replacement fibrosis; reduced from mag. × about 400.

Fig. 6.—This detailed autoradiogram was made from a section of the spleen that had been completely replaced with fibrous connective tissue. Note the numerous alpha tracks coming from the thorium dioxide aggregates; reduced from mag. × about 900.

Fig. 7.—Detailed autoradiograph of a splenic artery. Note the hyaline changes and the marked thickening of the wall. The thorium dioxide deposits and alpha tracks are seen in and around the blood vessel; reduced from mag. × about 650.

Fig. 10 (Patient A. D.).—Detailed autoradiograph of a central canal of a Haversian system. A small aggregate of thorium is deposited in the central canal along the superior border. Note the alpha track irradiating from the aggregate. There are five additional alpha tracks originating from the bone substance at the upper end of the central canal; reduced from mag. × about 850.





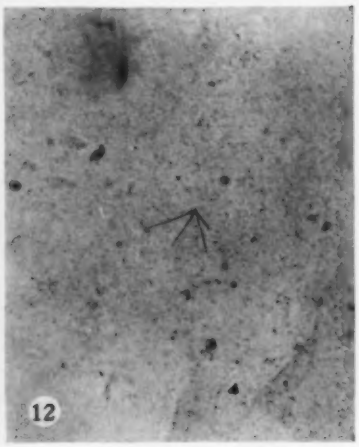


Fig. 8 (Patient S. L.).—Detailed autoradiograph of the cortex of the adrenal. Note the small particles of thorium dioxide and the alpha tracks in between the cells of the zona fasciculata; reduced from mag. × about 800.

Fig. 9 (Patient A. D.).—Photomicrograph of an autoradiograph of the bone marrow and endosteum of a rib. Aggregates of thorium dioxide and alpha tracks are present, adjacent to the endosteal surface. Note that a few alpha tracks, which are out of focus, are originating from the architecture of the bone; reduced from mag. × about 950.

Fig. 11.—A higher magnification of a thorium dioxide aggregate adjacent to the endosteum, showing the alpha tracks resulting from thorium decay; reduced from mag. × about 950.

Fig. 12 (Patient S. L.).—Photomicrograph of an autoradiograph, showing a four-pronged star in the compact bone of the femur; mag. × about 900.

splenic architecture with replacement fibrosis results in the contraction of the spleen. This increases the concentration of the irradiation dose, since the volume of the organ is reduced with little change in the thorium content. The progressive nature of the process has resulted in marked contraction of the spleen, demonstrated roentgenographically, in some patients having thorium dioxide for as long as 15 to 20 years.

Estimates of the radiation dosage delivered to the tissues may be made by quantitative autoradiography. Rotblat estimated the daily dose of irradiation from biopsy specimens from a patient who received 22 cc. of thorium dioxide in 1938, 11 years prior to operation. He estimated that the liver received 1.16 rep#

Roentgen equivalent physical.

per day; the spleen, 1.18; lymph node, 17, and tonsil, 105 by this method.[9]

Minor skeletal roentgenographic changes have been demonstrated in over 50% of a series of 17 patients who were recently evaluated and who have had thorium dioxide retained on an average of 16 years. These changes were found most often in the tibia and femur. There was thickening of the shafts with a longitudinal striation in some instances. In others, thickening of the trabeculae and/or irregularity of the endosteum was found. At present, no definite relationship has been established between the administration of thorium and the roentgenographic irregularities. The autoradiographs shown in this report demonstrate one possible mode of action. The ionizing radiation along the endosteal surfaces may be partially responsible for the irregularities demonstrated roentgenographically. The autoradiographic technique also confirms the deposition of thorium in the central canals of the Haversian systems. The presence of radioactivity in the skeleton may be from the deposition of thorium and/or from the transfer of one or more of the daughter radioelements of the thorium decay series from the major sites of deposition in the soft tissues. These radioelements are most likely the bone-seeking radium isotopes (mesothorium I and thorium X).

SUMMARY AND CONCLUSIONS

The eventual utilization of thorium as a breeder material in the production of atomic energy has made information about the effects of thorium dioxide (Thorotrast) one of considerable importance.

Material from patients who died 10 and 19 years after thorium dioxide administration has been studied by histological, autoradiographic, radiochemical, and spectrochemical means. These newer and more refined techniques give a better understanding of the distribution, rate of elimination, and mode of action of thorium and the members of the thorium decay series.

The deposition of thorium and/or the daughter radioelements of the thorium decay series in the skeleton and soft tissues is demonstrated by autoradiographic and histopathological means.

Problems relating to the calculations of the amount of irradiation received by the body and the various organs are discussed.

Drs. O. R. Farley, W. W. Eldridge, and K. H. Langenstrauss, of St. Elizabeth's Hospital, Washington, D. C., and Dr. G. M. Hass, Presbyterian Hospital, Chicago, made available the sections from patients, and Dr. M. M. Copeland, of Georgetown University; Dr. L. C. Johnson, of the Armed Forces Institute of Pathology, and Dr. J. A. Turner, of the Naval Medical School, aided in the interpretation of the histological sections. Mr. J. T. Stringer and Mr. J. E. McLean, of the Photographic Laboratory of the Naval Medical School, made the photomicrographs.

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Polyarteritis Nodosa in the Lung of a Newborn Infant

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Fibrinous arteritis, resembling polyarteritis nodosa, confined to the lungs is a rarity. Thus far, it seems always to have occurred in association with pulmonary hypertension. Thus, it has been described in association with mitral stenosis (Parker and Weiss, 1936; Spencer, 1950, and Weitzman and Husain, 1952), in association with pulmonary hypertension due to congenital heart disease (Old and Russell, 1950; Kipkie and Johnson, 1951, and Hicks, 1953), and in association with pulmonary hypertension of unknown etiology (Gilmour and Evans, 1943; Symmers, 1952, and McKeown, 1952).

The following case is of interest in that severe lesions similar to those of polyarteritis were found in the pulmonary arteries of an infant that died four hours after birth. As far as can be ascertained, there is no published report of arterial necrosis occurring in utero, although Wilmer (1945) describes an infant dying on the 10th day with severe umbilical sepsis and showing at autopsy generalized polyarteritis nodosa; and he concludes that the arteritis might have begun in utero.

CLINICAL HISTORY

Maternal History.—The mother, aged 31, had three previous normal pregnancies; these children are alive and well. There is no personal or family history of asthma, urticaria, hay fever, or allergic disease. During this fourth pregnancy her only illness was a single attack of common cold. She could not recall contact with any acute infectious disease. In September (at about 30 weeks' gestation) she had some colic, but no diarrhea, during an outbreak of dysentery which occurred in the institution where she lived. She received succinylsulfathiazole (Sulfasuxidine) as a prophylactic (the exact quantity or duration was not known), and, in spite of careful questioning, she was emphatic that no skin rashes or other toxic manifestations had developed during or after the administration of the drug.

At no time in her life had she received injections of serum or serum products, or been given a blood transfusion or any form of penicillin. She did not remember taking a sulfonamide on any previous occasion.

She denied having taken any self-medication, and, as far as could be ascertained, had not attempted to terminate the pregnancy by any self-administered drugs.

Radiation.—On two occasions radiograms were taken, a routine chest film at her first attendance (13 weeks' gestation) and one of the abdomen at 23 weeks' gestation. There was no history of industrial exposure to radioactive materials.

History of Present Pregnancy.—The last menstrual period commenced on Feb. 3, 1953. Routine antepartum examinations did not reveal any significant findings. The Kahn test was negative (this and the Wassermann reaction were again found to be negative three months after parturition). Her blood group was A, Rhesus-positive.

Labor commenced on Nov. 9 and normal delivery took place three and one-half hours later.

Infant's History.—The infant was deeply cyanosed and made little attempt to cry, in spite of gently physical stimulation. Mucus was sucked from the airway, nikethamide (Coramine) and lobeline injected into the umbilical vein, and an inhalation of a mixture of carbon dioxide and oxygen given. Cyanosis persisted and mucus was removed under direct vision. Physical examination showed poor air entry into both lungs; triple rhythm and splitting of the first heart sound were noted. The liver was enlarged to the umbilicus.

The child died 4 hours 20 minutes after birth and was still deeply cyanosed.

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Fig. 1.—Photograph of lung showing mottling of visceral pleura and prominent pulmonary arteries on the cut surface.

AUTOPSY FINDINGS

The autopsy was performed 11 hours after death.

The body was that of a normally developed male infant, weighing 9 lb. 9 oz. (4.3 kg.) and measuring 21½ in. (55 cm.) from heel to posterior fontanelle; the head circumference was 14 in. (36 cm.). There was marked cyanosis of the lips, skin, and nails and obvious edema of the occipital region of the scalp.

Body Cavities.—There was a straw-colored effusion in both pleural cavities; a pale yellow gelatinous coagulum over the visceral pleura was easily removed, revealing a slightly dulled pleural surface.

A similar effusion was present in the pericardial cavity; there was no ascites.

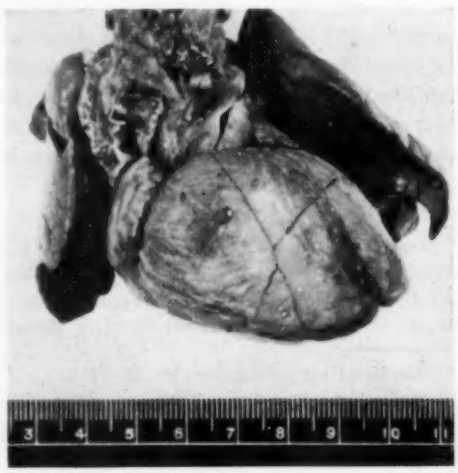
Respiratory System.—The larynx showed some edema of the epiglottis and vocal cords but was otherwise normal. The trachea and main bronchi were free from obstructing mucus.

The right lung weighed 30 gm., and the left, 32 gm. (after fixation); both were congested, purplish in color, and showed slight granularity of the pleural surface with occasional petechial hemorrhages. The cut surface of the organ was reddish in color, the bronchi were normal in appearance, but the pulmonary arteries stood out as thickened areas, giving the whole lung a mottled appearance (Fig. 1). All the lobes showed similar appearances.

Cardiovascular System.—The heart weighed 49 gm. after fixation as compared with a normal range of 14.5 to 17 gm. for infants of similar size and weight; it measured 45 mm. transversely, 60 mm. in a longitudinal axis, and 38 mm. anteroposteriorly (Fig. 2). The myocardium was firm and, on section, gross hypertrophy of both ventricles was noted, the left ventricle measuring 8 to 10 mm. in thickness, the right 10 mm., and the interventricular septum 10 mm. No other abnormality of the myocardium, pericardium, or endocardium was noted. The heart valves and great vessels were normal in anatomical arrangement. The ductus arteriosus was normal in position, size, and appearance and was patent; the foramen ovale was patent and of normal size. No atheroma was seen in the larger pulmonary arteries.

Alimentary System.—The esophagus, stomach, intestine, and pancreas seemed nor-

Fig. 2.—Photograph of heart and lungs showing generalized ventricular hypertrophy. Scale in centimeters.



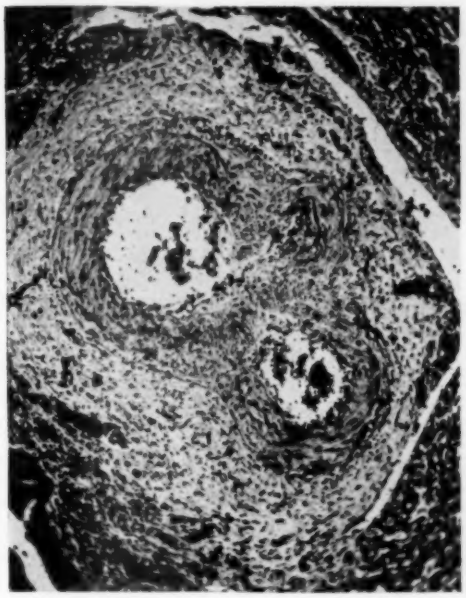
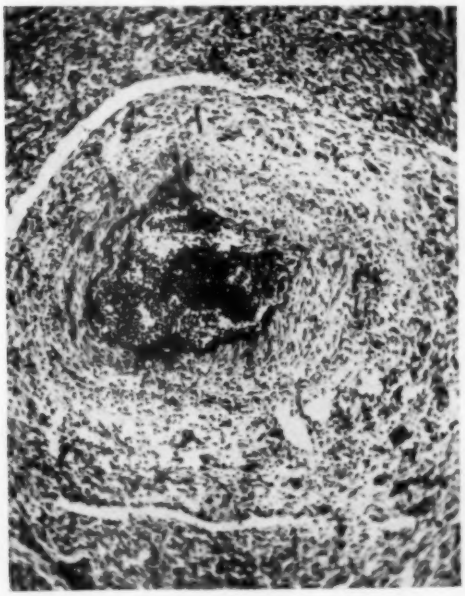


Fig. 3.—Pulmonary artery showing fibrinous change in media and chronic inflammatory cell infiltration of adventitia. This is typical of the milder forms of the lesion. Hematoxylin and eosin; reduced ⅕ from mag. × 130.

Fig. 4.—Severer change in media of pulmonary artery than that illustrated in Figure 3. Some early thickening of the intima is present. Hematoxylin and eosin; reduced ⅕ from mag. × 100.



mal. The liver was grossly enlarged and weighed 370 gm.; it was smooth and its cut surface was normal.

Nervous System.—Some small superficial tears and ecchymoses were present on each side of the attachment of the falx to the tentorium cerebelli, but no intracranial hemorrhage had occurred. The brain itself was normal.

Osseous System.—There was a small center of ossification in the lower epiphysis of the right femur, and the demarcation between it and the shaft was sharp.

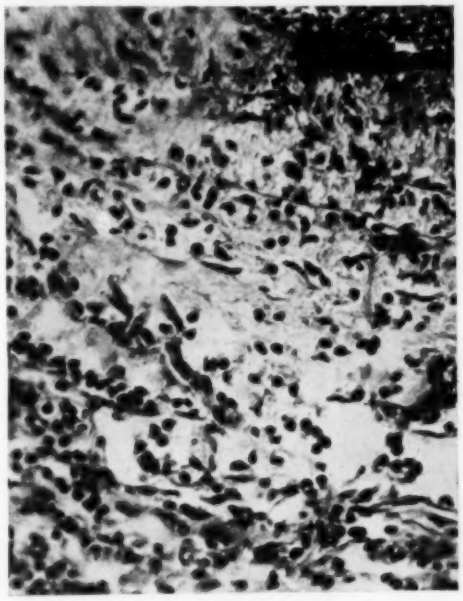


Fig. 5.—Higher power view of media and adventitia of same artery as that shown in Figure 4. The cellular reaction is composed of lymphocytes, histiocytes, some plasma cells, and only occasional polymorphonuclear cells. Hematoxylin and eosin; reduced ⅕ from mag. × 370.

The kidneys and spleen weighed 20 gm. each and were deeply congested. Otherwise all the remaining organs were normal.

HISTOLOGY

Tissues removed at autopsy were fixed in formol saline and sections stained with hematoxylin and eosin; selected sections were stained with phosphotungstic acid hematoxy-

lin, acid-picro-Mallory, Weigert's fibrin stain, Weigert's elastic stain, the periodic acid-Schiff technique, and silver impregnation for reticulin fibrils. Pieces of lung, pancreas, liver, and thymus were stained for spirochetes by Levaditi's method. Sections of lung were stained by Giemsa's and Mann's stain for virus inclusion bodies.

The lung parenchyma is normal but is incompletely expanded. The bronchi are normal and free from inflammation or obstructing mucus. An occasional alveolus and bronchiole contained meconium; this, however, is not widespread and is almost confined to the right lower lobe. The pleura shows minimal chronic inflammation, edema, and congestion.

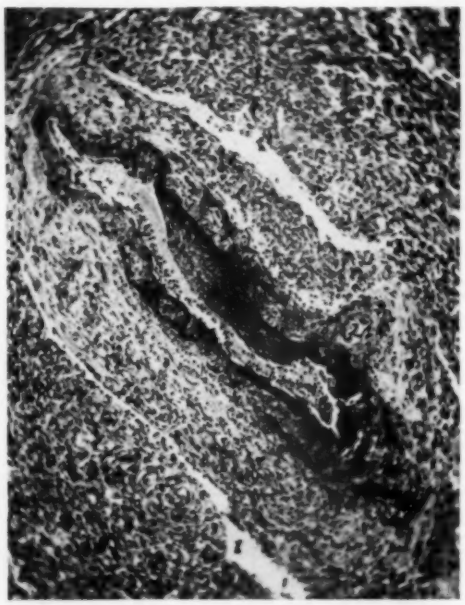


Fig. 6.—Severe fibrinous change in media of pulmonary artery with intimal thickening and marked chronic cell infiltration of adventitia. Hematoxylin and eosin; reduced ⅕ from mag. × 100.

The pulmonary arteries show striking changes affecting muscular branches of between 90μ and 300μ in diameter, with an average diameter of 160μ (measuring the approximate diameter of the internal elastic lamina). These changes consist of depositions of eosinophilic hyaline material in the

Fig. 7.—Moderately severe fibrinous change in media of pulmonary artery. The adjacent vein is free from such changes; some slight chronic inflammatory infiltration of its adventitia is present. Hematoxylin and eosin; reduced ⅕ from mag. × 100.

Fig. 8.—Same artery and vein as shown in Figure 7. The reticulin fibrils are intact in the affected areas; silver impregnation; reduced ⅕ from mag. × 100.

Fig. 9.—Same artery and vein as illustrated in Figure 7. The elastic laminae of the artery show thickening, flattening, and some fragmentation, while those in the vein are intact. Weigert's elastic stain; reduced ⅕ from mag. × 100.

media, varying in amount from small flecks to almost complete replacement of the whole of the media (Figs. 3, 4, and 6). This material stains blue with phosphotungstic acid-hematoxylin, red with picro-Mallory, and deep pink with the periodic acid-Schiff technique, although it failed to hold the Weigert's fibrin stain. Silver impregnation shows continuity of the reticulin fibrils through the areas of deposition (Fig. 8). Associated with this fibrinous change in the media the intima is edematous, fibrosed, and infiltrated with histiocytes and lymphocytes: Sometimes swelling of the vascular endothelium is evident. The internal elastic lamina remains outlined in these general destructive changes but is flatter than elsewhere and stains less sharply, often showing blurring of its outline and fragmentation (Figs. 9 and 10).

The adventitia is edematous and shows active fibroblastic proliferation. It is richly infiltrated with plasma cells, histiocytes, lymphocytes, and some polymorphonuclear cells (Fig. 5). Eosinophilic polymorphonuclears are present but not numerous. In some places the polymorphonuclears show pyknosis and karyorrhexis of the nucleus.

Often the lesion is restricted to part of the circumference (Fig. 11), and where the arteries are cut longitudinally this focal distribution is conspicuous.

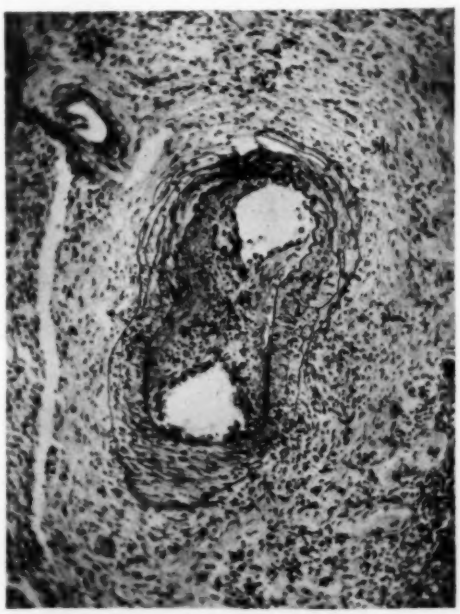
All the pulmonary arteries of this size range in the sections examined show some fibrinous change, although the degree of involvement varies.

In spite of the severe changes in the media, thrombosis was not observed. In an occasional vessel small mural plaques of fibrin were seen (Fig. 12), but these occupied only a fraction of the lumen.

The smaller pulmonary arterioles fail to show the intimal hyperplasia and medial fibrosis described in cases of pulmonary hypertension by Parker and Weiss (1936) and Symmers (1952).

The main pulmonary trunks are normal, apart from slight focal infiltration by lymphocytes and plasma cells around the vasa vasorum.

Fig. 10.—Showing marked loss of staining, blurring, flattening, and thickening of the internal elastic lamina in partially affected pulmonary artery. Weigert's elastic stain; reduced ⅕ from mag. × 100.



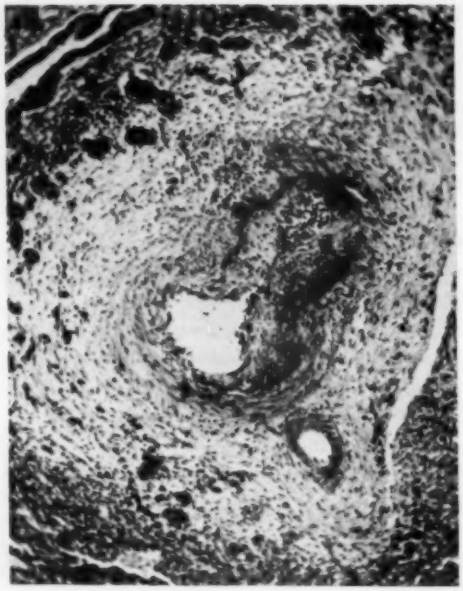
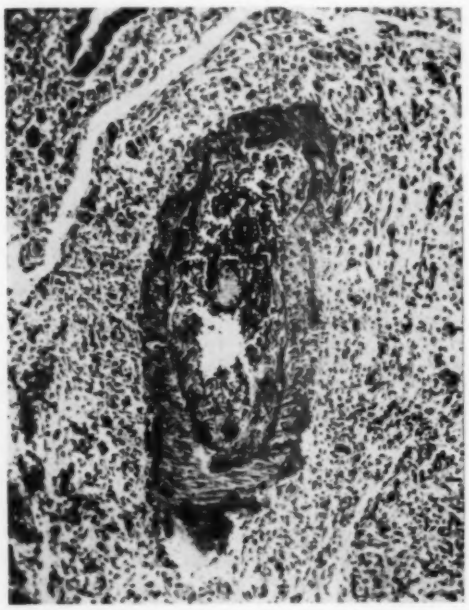


Fig. 11.—Same artery as shown in Figure 10. Only part of the circumference is affected. Comparison with Figure 10 indicates gross thickening of the intima over the affected media. Hematoxylin and eosin; reduced ⅕ from mag. × 100.

Fig. 12.—Severe fibrinous change in media of pulmonary artery with some fibrin plaques on the intima. These were the only evidence of thrombosis found. Hematoxylin and eosin; reduced ⅕ from mag. × 100.



The pulmonary veins are normal (Fig. 7). Unaffected arteries of the above size range, closely related to the bronchi and probably bronchial arteries, can be seen in one or two sections of the hilar regions of the lobes.

Systemic Arteries and Veins.—Apart from the pulmonary arteries, the only arterial abnormalities were found in the coronary and umbilical arteries. Altogether 16 blocks were prepared from the main coronary arteries and their larger branches; of these, 10 show arterial changes, which vary from slight focal intimal edema to marked intimal edema and

Fig. 13.—Coronary artery showing some intimal thickening and slight chronic inflammatory cell infiltration of media and adventitia. Hematoxylin and eosin; reduced ⅕ from mag. × 100.

some fibrosis affecting much of the circumference of the artery. This is associated with some loss of staining and fragmentation of the internal elastic lamina (Figs. 13 and 14), but no medial changes and no fibrin deposition have been seen. In the majority of sections the areolar tissue about the coronary arteries is sparsely infiltrated with lymphocytes. This infiltration is denser about those arteries showing lesions, and here some plasma cells are sometimes present.

The only lesion detected in the venous system was an infiltration by histiocytes and a few plasma cells and eosinophiles of the wall of a single vein of about 100μ diameter in the areolar tissue of the pancreas. There is no fibrin deposition in its wall (Fig. 15).

The myocardium from both ventricles and auricles is normal; it was not infiltrated with glycogen.

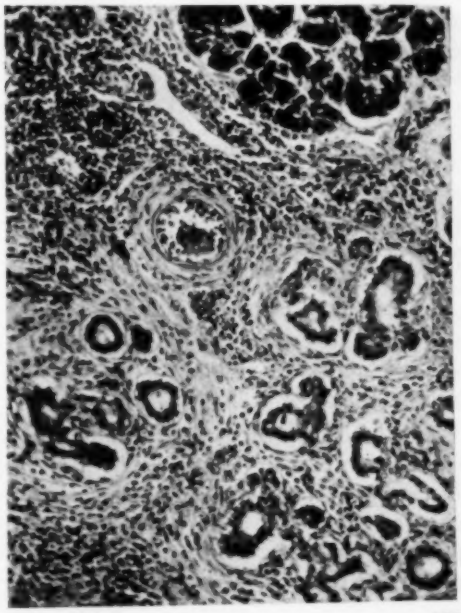
The pancreas shows foci of diffuse infiltration with lymphocytes, histiocytes, plasma cells, and some eosinophiles; these lesions

Fig. 14.—Coronary artery showing fragmentation of internal elastic lamina. Weigert's elastic stain; reduced ⅕ from mag. × 100.

reveal an early fibrotic reaction. Further fibrosis has occurred about the pancreatic ducts, and collagen has insinuated itself between the walls of adjacent alveoli and in places even between the individual alveolar cells. In some areas the alveoli have thus been completely destroyed and groups of ducts are seen lying in a zone of fibrous tissue (Fig. 16). There is no dilatation of the ducts elsewhere in the pancreas. Examination of serial sections shows that this inflammation is not the result of arterial degeneration in the neighborhood. The islets of Langerhans appear normal.

Fig. 15.—Vein from areolar tissue of pancreas showing moderate chronic inflammatory cell infiltration of its wall. Hematoxylin and eosin; reduced ⅕ from mag. × 82.

Fig. 16.—Pancreas showing chronic inflammation and fibrosis about ducts. Hematoxylin and eosin; reduced ⅕ from mag. × 162.



The liver contains many foci of hemopoiesis in the parenchyma and some in the portal tracts.

In the thymus there are small foci of fibrosis with infiltration by eosinophiles, lymphocytes, and scanty plasma cells.

The spleen shows congestion with foci of hemopoiesis in the pulp and an occasional focus of myelopoiesis in the trabeculae.

In the larynx there is edema of the submucosa and some congestion of the submucosal vessels. No evidence of inflammatory infiltration is seen.

A mediastinal lymph node shows hemopoiesis in the sinusoids.

The kidney is congested and contains an occasional focus of hemopoiesis.

The femoral epiphysis, tongue, and thyroid all show normal appearances.

The umbilical cord shows in general a normal structure, but both arteries have gross thickening of the intima with slight lymphocytic infiltration; no eosinophiles or histiocytes are present. Some fibroblasts arranged in radial fashion are present in the thickened intima. The elastic lamina is destroyed where the media is thinned by the thickened intima. This is quite different from the normal finding of thin-walled vessels with sharply defined elastic laminae. The umbilical vein shows a small area of intimal thickening with scanty lymphocytic infiltration.

COMMENT

This case of pulmonary arteritis in a newborn infant is of particular interest in that the changes, essentially similar to those of polyarteritis nodosa, affect the pulmonary arteries only. It seems unlikely that such obvious lesions could have developed within the four hours during which the lung was aerated, and we must regard this arteritis as having developed during intrauterine life.

It is probable that the inflammatory infiltration seen in a single vein in the pancreas is the result of the chronic inflammatory change in that organ and is not related to the pulmonary arteritis. Although the umbilical artery and vein show some degenerative and inflammatory changes, the impression was gained that it was not of the same nature as the pulmonary arteritis. The intimal thickening of the umbilical artery may, however, be related to the hypertension postulated below.

In an examination of the coronary arteries from stillborn infants, Levene * (1954) found in 9 out of 15 cases foci of splitting of the internal elastic lamina with or without thickening of the overlying intima. Dr. Levene examined sections of the coronary arteries from the present case and regards the splitting and fraying of the internal elastic lamina with the associated intimal thickening (Figs. 13 and 14) as an example of the lesion he has been studying, although more advanced than anything he had seen. These changes, which he considers may well be early atheromatous lesions, are therefore not likely to be the early changes of polyarteritis nodosa, as was thought during the early study of the present case.

ETIOLOGY

Focal arterial lesions have been reported in man in association with serum sickness (Eason and Carpenter, 1937; Clark and Kaplan, 1937; Rich and Gregory, 1943), with acute nephritis (Kipkie and Johnson, 1951), with sensitization to sulfonamides (Rich, 1942, and 1942a; More and co-workers, 1946), with asthma and allergic states (Bergstrand, 1946), and with asthma plus sulfonamide therapy (Smith, 1948). These would appear to be similar to, if not identical with, the lesions of polyarteritis nodosa unaccompanied by clinical evidence of sensitization.

Systemic arterial lesions similar to those of polyarteritis nodosa have also been demonstrated in malignant hypertension by Klemperer and Otani (1931) and in the pulmonary arteries in cases of pulmonary hypertension.†

* Levene, C. I.: Personal communication (1954).

† References listed in first paragraph of this paper.

Experiments designed to elucidate the etiology of polyarteritis nodosa fall into two categories: (*a*) the production of hypersensitivity to a foreign serum or to chemicals that presumably act as haptens (Rich and Gregory, 1943; More and McLean, 1949; Sheikh, 1951) and (*b*) the production of artificial hypertension in rats by renal ischemia (Smith and co-workers, 1944; Zeek and co-workers, 1948). Both procedures have resulted in arterial lesions similar to those of polyarteritis nodosa.

It is possible that in some cases of fibrinous arteritis there may be a synergistic combination of hyperergy and hypertension.

Hypertension.—It is difficult to imagine how a significant hypertension could be built up within the arteries of the unexpanded lung and, particularly, a hypertension confined to the pulmonary circulation of a fetus whose ductus arteriosus and foramen ovale were normal. Nonetheless, in this case gross hypertrophy of the muscle of both ventricles was present (the heart weighed almost three times the average weight for infants of similar size and was not infiltrated with glycogen or fat) and this at least suggests that pulmonary hypertension might have been present. The absence histologically of any hyperplastic changes in the smaller pulmonary vessels at first seemed to exclude that possibility until Professor Symmers (1954)‡ pointed out to me that he had failed to find them after prolonged study of postmortem material from a number of patients who were shown by direct measurement during life to have pulmonary hypertension.

In view of the general cardiac hypertrophy and the relative hypertrophy of the right ventricle in this infant, it is postulated that pulmonary hypertension existed during intrauterine life and played some part, at least, in localizing the arterial changes in the pulmonary circulation. The postmortem findings do not seem, however, to provide any explanation of the cause or causes that elicited this cardiac hypertrophy and hypertension.

‡ Symmers, W. St. C.: Personal communication.

Sensitization.—A hypersensitive state may be considered as the cause of the arteritis in this infant. One may postulate that an antigen was present in the mother, despite the fact that she had no personal or family history of hyperergic disease and had never received serum preparations, blood transfusions, penicillin, or sulfonamide, and that this hypothetical antigen passed the placental barrier into the fetus.

In reported cases of polyarteritis nodosa both the systemic and the pulmonary arteries and even systemic veins were affected (e. g., Rothstein and Welt, 1933; Friedberg and Gross, 1934; Harris and co-workers, 1939; More and co-workers, 1946); and in experimental hypersensitivity angiitis lesions were found in similar situations (Rich and Gregory, 1943). In contrast, Zeek and co-workers (1948) make the presence or absence of pulmonary arteritis a point of differentiation between vascular lesions due to hypersensitivity (hyperergic arteritis) and those due to hypertension. If this distinction is valid, the pulmonary localization of the lesions in this infant would seem to exclude hypersensitivity as a cause.

Polyarteritis nodosa has been described in association with sulfonamide sensitivity (Rich, 1942 and 1942a; More and co-workers, 1946; Bergstrand, 1946), the patients usually showing some intolerance to the drug at the time. While not excluding sulfonamide sensitivity as an etiological agent in this infant's arteritis, one finds it hard to conceive so severe arterial destruction resulting from a single, and apparently first, course of succinylsulfathiazole unaccompanied by any immediate toxic reaction. While the difficulty of demonstrating cutaneous sensitivity to the sulfonamides is freely admitted, the fact that the mother showed no sensitivity to both scratch and intradermal tests against succinylsulfathiazole six months later is probably further evidence against sulfonamide sensitivity as an etiological agent of her infant's arteritis.

Passage of antigens across the placenta has been proved experimentally. Thus, Rat-

ner and co-workers (1927) have shown that the guinea pig fetus can be sensitized to foreign proteins given to the mother, either parenterally or by feeding.

Infection.—Changes resembling those of polyarteritis nodosa have been described (Cairns and Russell, 1946) in the vasculature of the central nervous system in cases of pneumococcal meningitis surviving for seven or eight weeks. They suggest that sensitization by repeated reinfections of the cerebrospinal pathway from an unresolved focus may play a part in causing this type of arteritis. One must therefore consider the possibility of an inflammatory process as the cause of the arteritis in this infant.

The inflammatory reactions in the lung and more especially those in the pancreas are characterized by many plasma cells which are foreign to the newborn infant. This suggests a chronic inflammatory process, and syphilis was considered, particularly in view of the pericellular fibrosis in parts of the pancreas. This, however, was thought improbable in view of the negative maternal Wassermann and Kahn reactions (unfortunately, the father will not submit to a Wassermann). The failure to demonstrate spirochetes in the lung, pancreas, liver, and thymus is additional evidence against syphilis.

Whatever the nature of this infective process, it could have acted as a source of sensitization and thus acted synergistically with pulmonary hypertension in causing the arteritis in this infant.

It is now recognized that congenital abnormalities may result from acute infectious diseases, especially rubella, developing in the mother during pregnancy. This mother, however, did not suffer from any such disease, and she cannot recall contact with any patient suffering from such diseases.

Miscellaneous Considerations.—Incompatibility between maternal and fetal bloods is a possible cause of sensitization, but unfortunately the infant's blood group was not determined and the father will not agree to venepuncture.

It is unlikely that exposure to x-rays for only two diagnostic films would have produced such specific degenerative changes.

SUMMARY

Severe lesions similar to those of polyarteritis nodosa have been found in the pulmonary arteries of a newborn infant showing gross cardiac hypertrophy. Minimal lesions were found in the umbilical artery, the umbilical vein, and a single vein in the pancreas. Focal chronic inflammation with fibrosis was found in the pancreas and thymus. Changes indistinguishable from early atheroma were present in the coronary arteries.

Various etiological factors are discussed, and the combination of pulmonary hypertension of unknown cause with a sensitization due to chronic infection is suggested as a possible cause of the pulmonary arteritis.

Prof. A. C. Lendrum and Dr. A. G. Signy gave helpful criticism of the manuscript; Dr. I. M. Anderson granted permission to publish the clinical details; Mr. J. E. Andrews, Southern Group Laboratory, London, made the photographs, and Mr. R. A. Crawford, The Group Laboratory, St. Mary Abbots Hospital, London, prepared numerous sections.

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Experimental Hypertensive Vascular Disease in the Rat

A Histopathologic Study of the Lesions Produced by Methylandrostenediol and Desoxycorticosterone Acetate

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In a recent publication [1] it was reported that methylandrostenediol, an androgenic steroid of the 19-carbon series, produced hypertension, renal and cardiac hypertrophy, and morphologic changes in the vascular system, involving principally the kidney and heart. Similar lesions have been described by Selye [2] in suitably sensitized rats treated with desoxycorticosterone acetate and considered by him to be part of a generalized vascular phenomenon which he called the "hyalinosis syndrome." [3] A series of publications by Masson and co-workers * has dealt rather extensively with both the physiologic and the pathologic effects of treatment with anterior pituitary preparations, desoxycorticosterone acetate and cortisone, both alone and in combination with renin and angiotonin in the rat. These authors have described widespread morphologic changes in this species which, while differing somewhat in detail depending upon the particular experimental conditions, had sufficient similarities to warrant their being considered as manifestations of a generalized vascular reaction which they termed "hypertensive arteriolopathy."

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The vascular lesions which have been described by Goldblatt [8] in the dog with renal hypertension and those observed by Muirhead and co-workers,† Orbison, Christian, and Peters,[12] and Masson, Plahl, Corcoran, and Page [13] in the bilaterally nephrectomized dog bear striking similarities to those seen in the hypertensive rat under a variety of experimental conditions despite the species difference.

In the course of extensive study of the morphologic lesions produced by methylandrostenediol in the rat, it became apparent that many of the lesions described by the above authors under several experimental conditions were reproduced by treatment with this steroid in a single experiment. Since there were, in addition, interesting morphologic changes involving the adrenal cortex, it was considered worth while to present a comprehensive description of the morphologic alterations produced by this androgenic steroid and to compare them with the changes produced by desoxycorticosterone acetate under identical experimental conditions.

METHODS

Thirty female hooded rats weighing 83 to 100 gm. were unilaterally nephrectomized, given 1% sodium chloride to drink ad libitum, and divided into three groups of 10 rats each for the following treatment: Group 1 was untreated. The rats of Groups 2 and 3 received daily subcutaneous injections of 10 mg. of methylandrostenediol and 2 mg. of desoxycorticosterone acetate, respectively. Both steroids were administered as a microcrystalline suspension in 0.2 ml. of distilled water. The diet used was a balanced synthetic ration on which this strain of rats has been maintained for prolonged periods.[14] Observations were made on the body weight of the rats at weekly intervals throughout

† References 9 through 11.

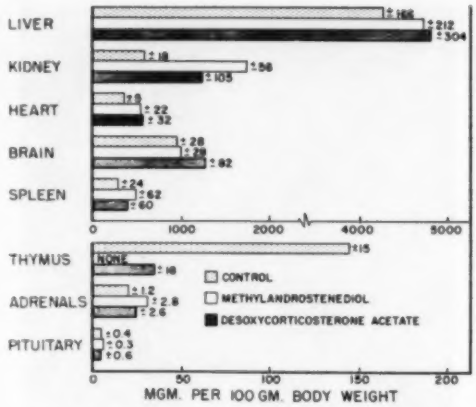
the experiment. The 1% sodium chloride consumption of each rat was measured daily and the systolic blood pressure determined at weekly intervals by the tail plethysmographic method,[15] with the rats under light ether anesthesia. At the conclusion of the experiment, the rats were killed by exsanguination, and the heart, kidney, adrenals, thymus, liver, spleen, brain, and pituitary were promptly removed. All tissues were fixed in Bouin's solution for 48 hours, dissected free of fat and connective tissue, and weighed on a chainomatic balance. Subsequently, microscopic sections were cut at 5μ and stained by the hematoxylin and eosin, McGregor's modification of the Heidenhain-Azan, periodic acid-Schiff, phosphotungstic acid hematoxylin, Verhoeff, Van Gieson, and Gomori aldehyde-fuchsin methods.

RESULTS

As previously reported,[1] hypertension developed in both groups of treated rats but differed in that the onset of hypertension in the rats receiving methylandrostenediol was more gradual and did not reach the high levels observed in the rats treated with desoxycorticosterone acetate. The changes in the 1% sodium chloride consumption of the experimental groups paralleled somewhat the blood pressure observations; i. e., the increase in consumption was slowly progressive in the rats receiving methylandrostenediol, while it was of rapid onset and of greater magnitude in those animals treated with desoxycorticosterone acetate. Only during the final week of the experiment did the 1% sodium chloride consumption of the two experimental groups approximate each other.

Gross Observations.—The average organ weights of the control and experimental rats, accompanied by the numerical standard error of the mean, are presented in the Graph.

(*a*) Methylandrostenediol-Treated Rats: The most striking observation was the greatly enlarged, pale kidney in all rats. The renal capsule stripped readily, showing a finely granular cortical surface with scattered small hemorrhagic spots and pale gray areas. The retroperitoneal tissues and the soft tissues about the pelvis of the kidney showed some edema. The hypertrophied hearts of all rats showed numerous gray areas over the epicardial surface. Although there was significant adrenal enlargement even on gross inspection, the most interesting observation was the nearly uniform presence of what appeared to be extensive focal adrenocortical hemorrhage. The brain was slightly enlarged and the convolutions were flattened, but no skull deformities were seen such as have been reported in young rats treated with desoxycorticosterone acetate.[3]



Average organ weights of unilateral nephrectomized, salt-treated rats administered methylandrostenediol and desoxycorticosterone acetate for six weeks. All plotted values are accompanied by the numerical standard error of the mean.

The thymolytic action of methylandrostenediol was demonstrated by the total absence of identifiable thymic tissue. In contrast, the spleens were significantly enlarged, but without gross morphologic changes. The pituitaries were of normal appearance. No lesions were seen in the vessels of either the pancreas or the mesentery of any of the animals.

(*b*) Desoxycorticosterone Acetate-Treated Rats: Although significantly hypertrophied, the kidneys of these rats were smaller and browner than those of the rats treated with methylandrostenediol and lacked the scattered hemorrhagic and pale gray spots on the cortical surface. There was, however, edema of the retroperitoneal and perirenal connective tissues. The cardiac enlargement was essentially identical to that observed in the rats receiving methylandrostenediol, and similar epicardial gray areas were evident. Significant enlargement of the brain with flattening of the convolutions was also pres-

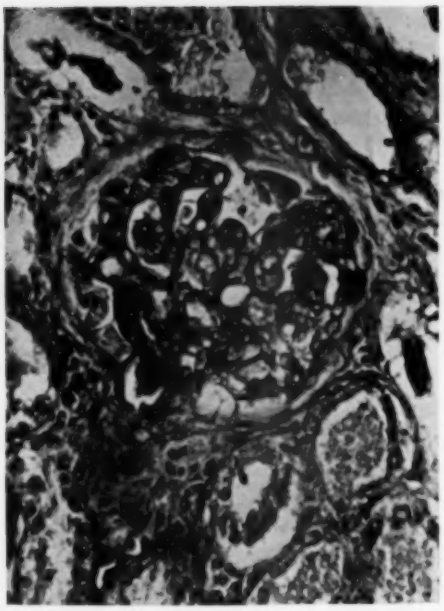


Fig. 1.—Glomerulus from rat treated with methylandrostenediol, showing a thrombus in a hilar capillary and periglomerular fibrosis. McGregor; × 310.

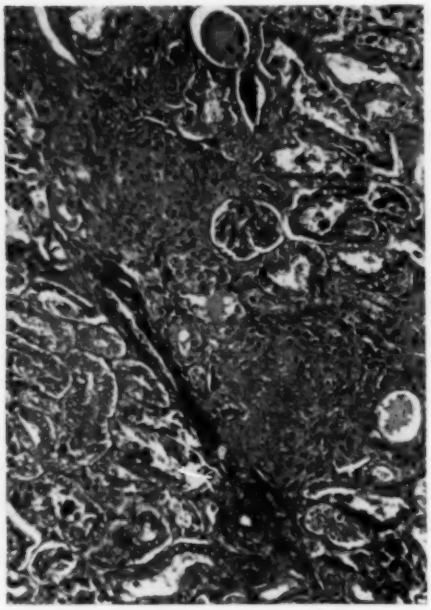


Fig. 3.—Virtually complete fibrosis of two glomeruli in the kidney of a methylandrostenediol-treated rat. Gomori aldehyde fuchsin; × 140.

Fig. 2.—Proliferation of parietal cells of Bowman's capsule and generalized capillary degeneration in a glomerulus of a rat treated with methylandrostenediol. McGregor; × 290.

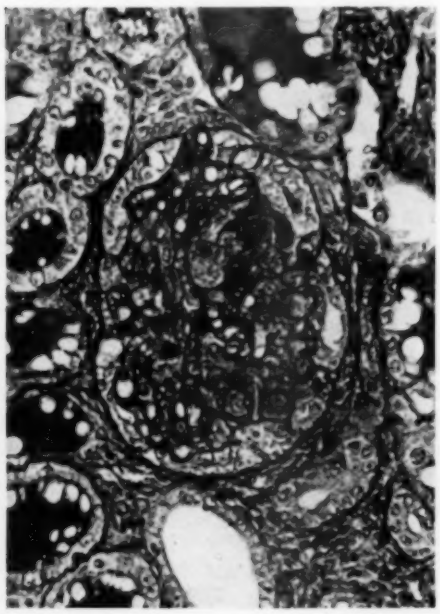
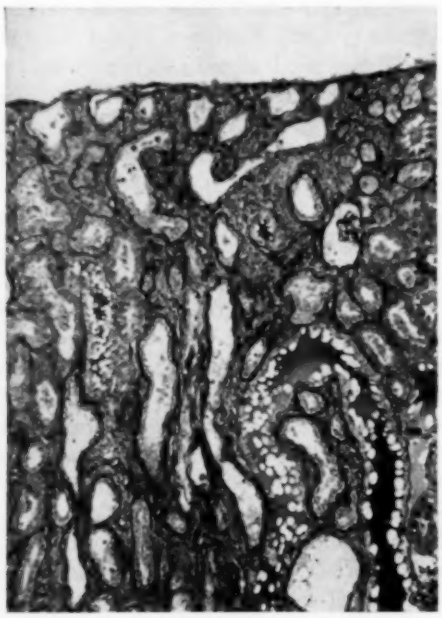


Fig. 4.—Dilated tubules filled with hyaline casts, thickened basement membranes, and slight interstitial fibrosis in a rat receiving methylandrostenediol. McGregor; × 100.

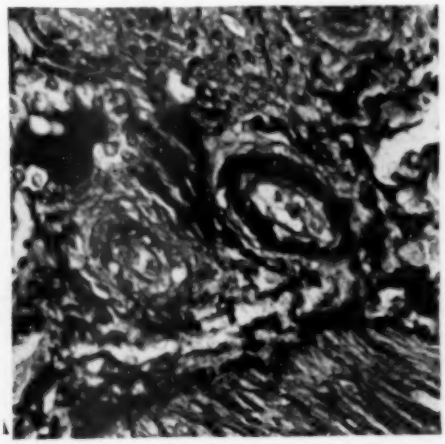


ent in these rats. The adrenals were neither enlarged nor otherwise unusual in gross appearance. Thymic tissue was present in all rats of the group, although of quite variable quantity, and the spleen was slightly enlarged. No peripheral vascular lesions were seen grossly.

Microscopic Observations.—(*a*) Methylandrostenediol-Treated Rats: 1. Kidney. In the kidney, as in other organs, there was considerable variation in the severity of the lesions from animal to animal as well as from area to area within the same slide. This biological variation in the severity not only allows a more detailed morphologic description of the lesions but also makes possible some concept of their pathogenesis.

Glomerular lesions, although focal, constituted an important part of the renal morphologic changes. Generally they were larger and more cellular than normal, with proliferation of both endothelial and epithelial cells (Fig. 1) and dilatation of capillary loops of the glomerular tuft (Fig. 2). The capillary basement membranes appeared swollen and indistinct (Fig. 2), with frequent separation of the capillary and parietal components by intramural masses of material which stained positively in the periodic acid-Schiff procedure, pink with eosin, brilliantly red in the McGregor and Gomori stains, and purple with phosphotungstic acid hematoxylin. The extent of glomerular tuft involvement varied from small focal intramural masses which formed a "cap" on a capillary loop to complete encirclement of the capillary with extension into the connective tissue of the mesangium. It is material with tinctorial characteristics such as this that has been called "hyalin" or "fibrinoid" in nature, and for purposes of the present description will be referred to as "fibrinoid material" or to represent a "fibrinoid change or alteration." Material with these tinctorial characteristics was also present in glomerular spaces and within capillary lumina (Fig. 2) and was interpreted as extravasated material in the former locations and fibrin thrombi in the latter. Occasional glomeruli showed "fibrinoid changes" with thickening and fragmentation of the capsular basement membrane and periglomerular fibroblastic proliferation. Severely involved glomeruli showed extensive break-down of the capillary tuft with masses and droplets of the same "fibrinoid material" within the glomerular space or within the cytoplasm of epithelial cells. Complete fibrosis of glomeruli such as that depicted in Figure 3 was also present. It should be

Fig. 5.—Arteriole with fibrinoid changes of moderate degree limited to the media. Rat administered methylandrostenediol. McGregor; × 310.



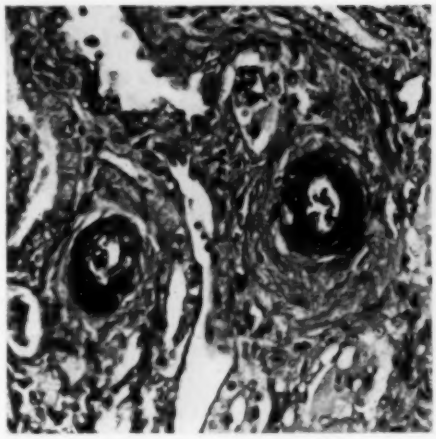


Fig. 6.—Two arterioles showing fibrinoid necrosis. In one the fibrinoid change appears limited to the media, while in the other the adventitia and intima are also involved. Note absence of elastic lamellae in arteriole on the right and partial degeneration of internal elastic lamella in arteriole on the left. Rat treated with methylandrostenediol. Gomori aldehyde fuchsin; × 270.

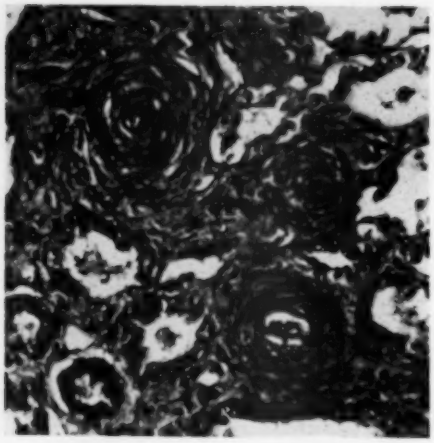


Fig. 7.—Three arterioles showing connective tissue proliferation in their walls with degeneration of elastic lamellae, narrowing of the lumina, and endothelial swelling in a rat treated with methylandrostenediol. Gomori aldehyde fuchsin; × 260.

noted that, despite the extensive glomerular degeneration, hemorrhage was infrequently observed.

Tubular changes consisted of prominent dilatation with segmental thickening and fragmentation of the basement membranes, flattening of the epithelium in the proximal convoluted segment (Fig. 4), focal basophilia of epithelial cells, and areas in which large, pale cells concentrically filled or projected into the tubular lumen. Many tubules contained casts which stained variously periodic acid-Schiff-positive or -negative, deep red to blue in the McGregor and Gomori stains, and gray-orange to deep purple with phosphotungstic acid hematoxylin, and cytoplasmic droplets of similar material were present in tubular epithelial cells, the latter frequently having desquamated into the lumen. Rarely was droplet formation observed in tubular cells without the simultaneous presence of luminal casts, an association which suggests that the intracellular droplets represent reabsorbed material from the tubules.

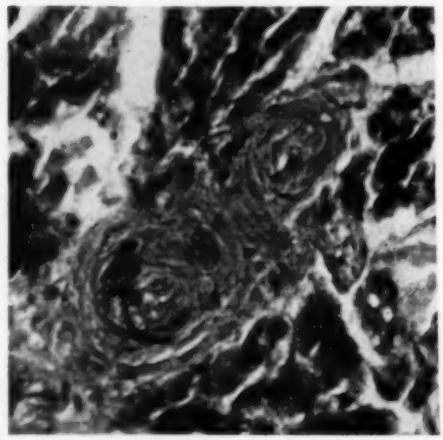
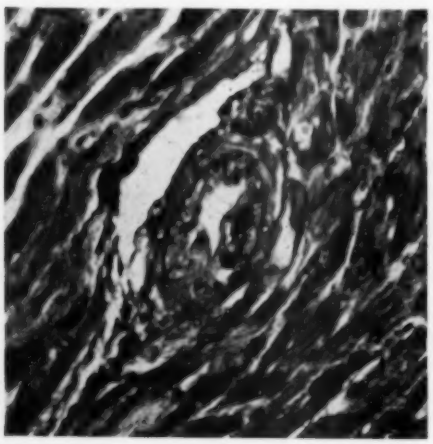


Fig. 9.—Myocardial arterioles with fibrinoid medial necrosis, loss of elastic lamellae, and endothelial proliferation. Rat treated with methylandrostenediol. Gomori aldehyde fuchsin; × 330.

Fig. 8.—Myocardial arteriole containing fibrinoid material in the intima which has been thickened by connective tissue proliferation. Rat treated with methylandrostenediol. Gomori aldehyde fuchsin; × 330.



Vascular changes other than those in the glomeruli were limited to the arterioles and small arteries (Figs. 5 to 7). In the larger arteries the individual muscle cells showed a homogeneous "fibrinoid change" in the sarcoplasm, which stained brilliantly red in the McGregor and Gomori stains and purple with phosphotungstic acid hematoxylin. In smaller arteries and arterioles such material was seen to completely encircle the vessel limited to the media, or sometimes extending to adventitia or intima as well. Other vessels showed proliferation of the adventitial connective tissue, degeneration and fragmentation of the external elastic lamella, and vari-

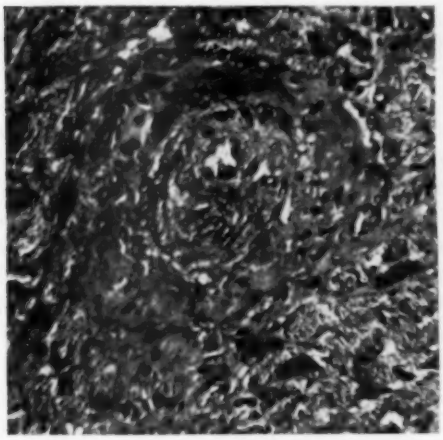


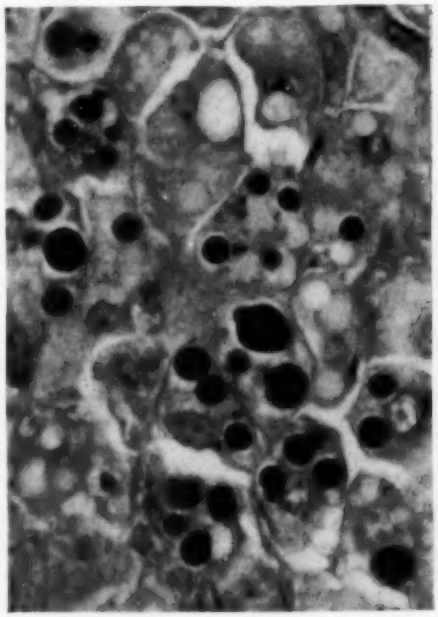
Fig. 10.—Myocardial arteriole showing sclerosis of all layers of the wall with endothelial swelling and residual fragments of the internal elastic lamella. Note thickness of the wall and narrowing of the lumen. Rat treated with methylandrostenediol. Gomori aldehyde fuchsin; × 330.

able replacement of the media by connective tissue. The internal elastic lamella of some arterioles was often partially or completely absent, and the intima was so thickened by collagenous connective tissue and endothelial hyperplasia that the lumen was markedly narrowed. Partial or complete occlusion of arterioles was produced by thrombi having the same staining characteristics as the "fibrinoid material" in glomeruli and vessel walls. The walls of veins were occasionally involved by the interstitial fibroblastic proliferation about glomeruli or arterioles, with resultant formation of overlying mural thrombi.

2. Heart. The small arterioles of both atria and ventricles were extensively involved by changes such as those described in the kidney. These varied from the presence of only "fibrinoid material" within the intima, media, or adventitia to include endothelial hyperplasia, focal degeneration and splitting of the elastic lamellae, connective tissue thickening of the intima, fibrous replacement of the media, and perivascular fibroblastic proliferation (Figs. 8 to 10). Areas of myocardial degeneration and fibrosis, when adjacent to the epicardium, undoubtedly accounted for the gray spots observed grossly.

3. Adrenal. The fibrous capsule was thickened, and there was extensive sclerosis and "fibrinoid change" of the periadrenal arterioles. The inner zones of the cortices showed necrosis and fibrosis, which varied from small foci to areas which involved almost the total cortex. The glomerulosa did not appear especially atrophic. The most consistent morphologic finding was the presence of one or two large or multiple small vacuoles in the cytoplasm of the cells in the inner two zones with displacement of the nucleus to one side, so that the cell resembled a fat cell. Associated with this change was the presence of intracytoplasmic "colloid" globules (Fig. 11), which varied in number from a single large one to several small globules occasionally appearing to fit into the vacuoles which were also present within these cells. These peculiar intracellular bodies stained uniformly reddish-pur-

Fig. 11.—Vacuolation of the cytoplasm of the cells of the zona fasciculata of the adrenal cortex accompanied by numerous periodic acid-Schiff-positive, intracytoplasmic globules in a methylandrostenediol-treated rat. Periodic acid-Schiff; × 800.



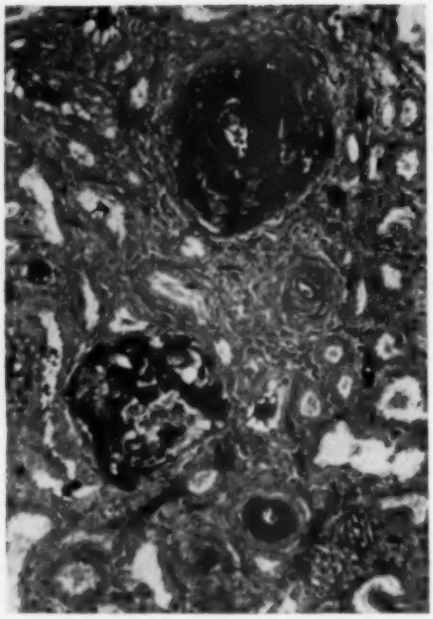


Fig. 12.—Two glomeruli showing different degrees of glomerulonecrosis accompanied by one arteriole showing fibrinoid change of the media and another showing replacement of the media by connective tissue. Rat treated with desoxycorticosterone acetate. Gomori aldehyde fuchsin; × 160.

ple in the periodic acid-Schiff stain, green in the Gomori stain, blue in the McGregor stain, and brownish-orange in the phosphotungstic acid hematoxylin stain. While the globules were present within the cells in intact regions of the cortex, it is noteworthy that they also occurred in degenerating cells within areas of necrosis, although they did not appear to be more numerous in these regions than elsewhere.

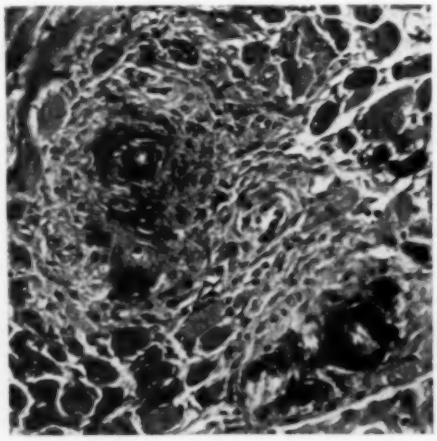
4. Other Tissues. Minimal vascular changes similar to those described for the renal and cardiac arterioles were observed in the small arteries of the pancreas and liver. The hepatic lobules showed a slight degree of central congestion and atrophy of the hepatic cell cords. In the spleen the lymphoid follicles were prominent, there was sclerosis of the central arterioles, and the sinusoids were congested. No lesions were seen in the brain.

(*b*) Desoxycorticosterone Acetate-Treated Rats: 1. Kidney. The renal lesions in this group were qualitatively the same as those observed in the rats treated with methylandrostenediol but quantitatively severer. Fewer glomeruli showed morphologic changes than in the animals treated with methylandrostenediol, but the lesions present were generally more pronounced (Fig. 12). The tubules of affected nephrons were only slightly dilated, and filled with hyaline casts, and the basement membranes were thinner and less fragmented. Interstitial fibrosis was distinctly less than that observed in the rats of the previous group.

The vascular lesions were also severer, particularly in the small arteries where medial smooth muscle degeneration was prominent and appeared as red smudges in the McGregor and Gomori stains, as purple masses in the phosphotungstic acid hematoxylin preparations, and were reddish-purple in the periodic acid-Schiff stain. The external elastic lamella tended to be fragmented and dispersed in a perivascular adventitial fibroblastic proliferation in the larger vessels, and both elastic lamellae were unrecognizable in small vessels showing severe "fibrinoid change" (Fig. 12).

2. Heart. The vascular lesions in the heart were very similar to those in the

Fig. 13.—Arterioles in the myocardium of a desoxycorticosterone acetate-treated rat showing the intense fibrinoid change of their walls with similar material in the adventitia associated with fibroblastic proliferation and scarring. Gomori aldehyde fuchsin; × 240.



kidney, consisting of various degrees of "fibrinoid necrosis," endothelial hyperplasia, and connective tissue replacement of their walls (Fig. 13). The elastic lamellae were poorly defined, especially in the more severely involved vessels, and the internal elastic lamella frequently showed abortive attempts at reduplication. Several hearts showed organized mural thrombi overlying areas of myocardial fibrosis, and one heart contained an organizing ball thrombus attached to the endocardium of the left atrium.

3. Adrenal. The capsule was thinner than in the rats of the previous group, and the pericapsular vascular changes were much less pronounced. Small foci of necrosis were detected in the cortices of a few rats, but no colloid globules were present within the cytoplasm of cortical cells. The zona glomerulosa was well defined and appeared slightly thinner than in the rats treated with methylandrostenediol.

4. Other Tissues. Changes in other organs and tissues did not constitute an important part of the pathologic findings. Slight vascular sclerosis and necrosis were present in pancreatic arterioles, but no lesions such as the periarteritis or panarteritis described by Selye [2] and Masson and co-authors ‡ were observed in the mesentery under the present conditions. Cerebral edema was more evident in these rats than in those of the previous group, and occasional meningeal arterioles showed slight "fibrinoid change."

COMMENT

The underlying defect in the "hyalinosis syndrome" [2] has been thought to be a change in the permeability of the vessel wall consequent to membrane degeneration.[16] Hence the lesions were considered to result from infiltration of a substance from the blood into the vessel wall and beyond, with subsequent degeneration and fibroblastic proliferation resulting in what has been called fibrinoid degeneration or necrosis. It should be recalled that a similar pathogenetic concept of acute vascular necrosis in malignant hypertension in man had been proposed by Schürmann and MacMahon,[17] who suggested that the basic lesion involved a break in the endothelium which if mild allowed the passage of plasma into the wall and if severe allowed the extrusion of red and white blood cells also.

On the other hand, Muirhead and his co-investigators § have described fulminant vascular lesions characterized by necrosis and smudging of the smooth muscle of the media of small arteries and arterioles in bilaterally nephrectomized dogs maintained alive for only a few days, but when survival was prolonged for periods of 10 to 20 days, the lesions consisted of frank medial necrosis with nuclear pyknosis or karyorrhexis and a tendency toward hyalinization. If survival exceeded 20 days, the vascular changes were characterized by narrowing and eccentricity of the lumen, subendothelial accumulation of connective tissue, hyaline substance or both, thickening of the internal elastic lamella associated with atrophy and disappearance of the smooth muscle of the media, hyalinization of the media, and finally connective or fibrous tissue replacement of all layers of the wall. These authors have expressed the opinion: that "it seems unlikely that the smudged material represents protein forced into the vascular wall through the endothelial lining which consistently shows the same staining characteristics as smooth muscle with multiple staining techniques," and have suggested, therefore, that "fibrinoid" is derived from necrotic smooth muscle of the media and can, under different circumstances, "flow" either toward the lumen or toward the adventitia.

While the present experiment does not constitute a temporal study, it is felt that lesions in which "fibrinoid" changes form a prominent component are the counterpart of those acute lesions described in rats treated with desoxycorticosterone acetate and renin,[5] cortisone and renin,[6] and desoxycorticosterone acetate and angiotonin,[7] as well as the acute lesions observed in bilaterally ne-

‡ References 4 through 7.

§ References 9 through 11.

phrectomized dogs.‖ On the other hand, the vascular lesions showing connective tissue proliferation in the adventitia, concentric replacement of the smooth muscle of the media, and degeneration of the elastic lamellae seem to correspond closely to the "chronic" lesions described by Muirhead, Turner, and Grollman [11] in bilaterally nephrectomized, hypertensive dogs maintained alive for periods longer than 20 days. The predominant vascular lesion in the present experimental material was of a combined type exhibiting features of both the "chronic" and the "acute" reactions of the latter authors and suggests that medial degeneration and protein transudation may play successive roles in their pathogenesis. It is my opinion that present evidence is inadequate to permit any definitive conclusions, and subsequent experiments are being designed to investigate this aspect of experimental hypertension in greater detail.

In general, the glomerular lesions were the same as those previously described in rats treated with lyophilized anterior pituitary substance,[18] growth hormone,[19] desoxycorticosterone acetate,[4] desoxycorticosterone acetate and renin,[5] desoxocortisone,¶ cortisone alone, and cortisone and renin,[6] and in rats with unilateral constriction of the renal artery # or silk perinephritis.[4] While the principal glomerular lesions consisted of capillary dilatation and thickening and fuzziness of basement membranes with "fibrinoid change" of the capillary wall and fibrin thrombi within capillary lumina, more severely involved glomeruli showed narrowing of capillary lumina and swelling of endothelial cells, fusion and loss of structural integrity of capillary loops and material in the glomerular space having similar tinctorial characteristics to that in the capillary walls and in the fibrin thrombi. Such glomerular changes have been variously considered in the literature as hyalinization [2] or necrotizing glomerulitis.[4] However, because the glomerular lesions in the present type of experimental hypertension, as well as those observed in other types of experimental hypertension, and in human malignant hypertension, are characteristically of a degenerative nature, it is felt that the best designation would be "glomerulonecrosis." Since afferent arterioles in the kidney often show complete or partial occlusion either by fibrin thrombi or by narrowing of the lumen consequent to the vascular changes, it seems reasonable to suggest that the glomerulonecrosis may be entirely secondary to the lesions involving the afferent arteriole rather than a specific glomerular degeneration.

The focal atrophy of myocardial fibers and replacement fibrosis in the heart as well as the increased interstitial fibrosis in the kidney attest to both the chronicity and the obliterative character of some of the vascular lesions. When either renal veins or endocardium were involved by this interstitial scarring, overlying mural thrombi were formed similar to those occasionally observed in malignant hypertension in the human being. The cytoplasmic vacuolation of the cells of the zona reticularis and zona fasciculata are characteristic of the "fatty metaplasia" which has been described by Selye, Rowley, and Hall,[24] and Selye and Stone,[25] in rats treated with methyltestosterone. However, the additional presence of intracytoplasmic "colloid" globules in the adrenal cortex of rats receiving methylandrostenediol had not been observed until recently [1] to follow androgen administration alone. The association of colloid globules with cortical necrosis was not constant, since they were frequently present in areas which did not show evidence of necrosis and were entirely absent from regions with advanced necrosis. This, of course, does not militate against their being evidence of early necrobiosis and, indeed, such globules have been observed in rats by Wilbur and Rich [26] under conditions in which "tubule" or "lumen" formation in the adrenal cortex was produced by massive acute overdosage with adrenocorticotropic hormone. Furthermore, I have observed such intracytoplasmic globules in human autopsy material, most commonly

‖ References 9 and 10.
¶ References 20 and 21.
# References 22 and 23.

in association with additional evidence of increased adrenocortical function. Nonetheless, the nature of these "colloid" globules remains unknown, but they are apparently of glycoprotein character, since they stain brilliantly red with the periodic acid-Schiff technique and blue in the McGregor stain.

SUMMARY AND CONCLUSIONS

Hypertension and concomitant vascular lesions principally involving the kidneys and heart have been produced by methylandrostenediol in the unilaterally nephrectomized rat given 1% sodium chloride to drink. Comparative histopathologic study of the vascular lesions produced by methylandrostenediol and those produced by desoxycorticosterone acetate has revealed their essential similarity. These lesions are felt to represent both chronic and acute vascular reactions, the former consisting of progressive degeneration and connective tissue replacement of mural components resembling that described in bilaterally nephrectomized hypertensive dogs maintained alive for more than 20 days, while the latter consists of a "fibrinoid" alteration of vessel walls similar to that observed in bilaterally nephrectomized, hypertensive dogs living less than 10 days. Glomerulonecrosis with fibrin-like thrombi occluding afferent arterioles and capillary tufts is considered part of the acute vascular reaction and is associated with degeneration of basement membranes.

While peripheral arterial lesions have not been a prominent feature of the changes produced by methylandrostenediol, arteriolonecrosis of the periadrenal arterioles has been consistently observed together with necrosis of adrenal parenchyma. Vacuolation of cortical cells in the zona reticularis and zona fasciculata associated with intracytoplasmic "colloid" globules was also observed in the adrenal cortex of rats treated with methylandrostenediol, but not in the adrenal glands of rats receiving desoxycorticosterone acetate. The glycoprotein nature of the globules has been suggested by their markedly positive reaction in the periodic acid-Schiff technique, but whether they represent a change in cytoplasmic protein, phagocytosed transudate from damaged arterioles, or storage of secretion material is not known.

The pathogenesis of the vascular lesions has been discussed relative to the theories that "fibrinoid" represents either an intramural protein transudation or an autochthonous degeneration of the smooth muscle of the media. The concept has been presented that both medial degeneration and transudation may result in the characteristic morphologic appearance of what has been termed "fibrinoid necrosis."

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Mural Fibrin Thrombosis of the Descending Aorta

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Although mural thrombosis in major vascular channels is a well-known entity, particularly as it relates to the chambers of the heart, the arteriosclerotic abdominal aorta, and syphilitic aneurysms of the ascending aorta and major tributary arteries, the pathological entity and clinical sequelae of massive mural fibrin thromboses of the otherwise undamaged descending aorta is little recognized or appreciated. The major significant observations related to this entity have been made by Rokitansky,[1] Duguid,[2] and Rannie and Duguid [3] as corollaries to their studies on the pathogenesis of atherosclerosis. Following the basic thesis of Rokitansky's "encrustation" hypothesis, Duguid considered minute fibrin thrombi on the wall of the aorta or deposition of cholesterol-bearing cells on the intima of vessels to be the earliest anatomical observations in the formation of atheromata. McLetchie [4] attempted to duplicate the Duguid lesion experimentally, but with no success. In his discussion of thrombogenic atheromata, the latter states that the "role of thrombogenesis must stand or fall with the question of microscopic coagula," although he does not seriously consider that atheromata are "commonly developed by episodes of relatively gross thrombosis."

This report considers three instances of massive mural fibrin thrombosis of the descending aorta of varying ages and the results thereof. The observations in these cases lead us to believe that (*a*) there is an anatomically definable disease of mural fibrin thrombosis of major vessels, (*b*) the thrombotic disease is not causally related to any intrinsic abnormality of the wall of the vessel, although the fortuitous existence of certain abnormalities of structure offers preferential sites of localization for the thrombi, and (*c*) the lipidization found in association with old fibrin thromboses can be clearly separated from other types of atheromatous vascular lesions.

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REPORT OF CASES

CASE 1 (DCGH 53-A-419).—*Clinical History.*—E. H., 38-year-old Negro male patient, presented signs and symptoms of acute arterial insufficiency in both lower extremities of about two days' duration. Aortography demonstrated an incomplete block at the level of the first lumbar vertebra. A saddle embolus at the aortic bifurcation with extension into both iliac arteries was removed surgically, but the patient died seven hours after his operation. The patient's significant past history included a transient right hemiplegia with aphasia, at the age of 34. At the same time he received a full course of antisyphilitic treatment.

Gross Autopsy Findings.—The body was that of a well-developed and well-nourished young man. The only significant pathology was found in the cardiovascular system and the brain. The heart

had no gross abnormality other than slight fusion of some of the chordae tendineae of the mitral valve. There was no other evidence of valvular disease, nor were any thrombi or vegetations found. The aorta had a normal degree of elasticity and displayed no dilatation or other structural abnormality. The intima was entirely free of atheromata except for minimal fine yellow streaking in the ascending thoracic segment. In the descending thoracic segment, immediately below the arch, the aortic lumen was compromised by a roughly ovoid mass, 1.5 cm. in greatest diameter, firmly attached to the intima ventrally. The mass consisted of firm, friable thrombotic material marked by alternating lines of yellow and pink. Near the mass, the intima had a minute degree of longitudinal and transverse wrinkling. The aortic wall underlying the thrombotic mass had no abnormality of structure, nor did any para-aortic structure appear abnormal. At the aortic bifurcation, the surgical incisional wound was closed and the intima in this area was covered by a thin layer of material similar to that in the upper mass. An occlusive clot extended from the bifurcation of the right common iliac artery into the internal and external branches, differing from the mass in the thoracic aorta by its tenuous attachment to the intima. The rest of the vascular tree was unremarkable. The brain had a 3 cm. pyramidal area of encephalomalacia with cavitation in the left parietal lobe. No vascular occlusion was demonstrated. The remainder of the organs displayed no gross abnormalities.

Microscopic Findings.—The major thrombotic mass in the thoracic segment of the aorta was composed almost exclusively of interlacing strands of fibrin, the material between the strands being an amorphous and finely granular precipitate. The fibrin strands were finer toward the lumen and wider toward the base, appearing to be undergoing a process of coagulation. The outer layer of fine strands was identified as fibrin by phosphotungstic acid hematoxylin stains. The wider inner bands of material stained by this method had the same character, except for the appearance of occasional unstained vacuoles within the mass similar to those described by Pagel as "fibrinoid" material. At the base, there was an incorporation of the fibrin into the intima and subintima of the aorta. Very rare fine clefts were present in the subintima, suggesting a fine, but sparse, cholesterol deposit. The outer third of the media beneath the thrombus was vascularized by capillaries, the lumina of which contained a moderate number of polymorphonuclear leucocytes and lymphocytes. There was no medial degeneration, but, rather, a separation of the medial muscle cells and elastic fibers by the capillaries. The adventitia was not abnormal, and the adventitial vessels had no stigmata of syphilis.

Distal to the major thrombus, a microscopic mass of fibrin of the same structure as described above was found lying on a completely unaltered aortic wall. This mass was not incorporated into the intima and subintima at its base.

The occlusive structure in the iliac vessels and the surgically removed portion of the saddle embolus were of the same nature as that of the major thoracic mass.

COMMENT

The lesions in this case are apparently acute mural fibrin thrombi on an aortic wall forming a lesion from which a saddle embolus originated. The alterations of intima or of luminal diameter usually associated with aortic thrombosis are absent. No anatomical evidence of arteriosclerotic or syphilitic aortitis was found. The capillary vascularization of a portion of the outer third of the media underlying the fibrin mass may be interpreted as either antedating the thrombosis or secondary to the thrombosis. If the latter case is true, it is the first anatomical evidence of reaction to and organization of the mass. Because of the lack of any adventitial change, the latter interpretation is the most likely one.

CASE 2 (MSH #15196).—*Clinical History.*—E. M., 46-year-old Negro woman, presented in shock with signs and symptoms of an acute abdominal catastrophe, localization being impossible. An abdominal exploration was performed, revealing infarction of most of the small bowel and discoloration of the stomach and part of the large bowel. No resection was attempted. The patient died several hours after her operation.

Gross Autopsy Findings.—The esophagus was markedly congested. The stomach, small bowel, cecum, and ascending colon were edematous, congested, and discolored, with patchy necrosis of the mucosa throughout these organs. The transverse

Fig. 1 (Case 1).—Mural fibrin thrombosis of aorta, acute.

Fig. 3 (Case 1).—Fibrin meshwork composing the thrombotic material on the aortic wall. There is one small focus of fibrin coagulation.

colon was congested and edematous but not necrotic. The descending colon was normal. The peritoneum was mottled a red-green color, and 80 cc. of dark bloody fluid was present in the abdominal cavity. The mesenteric vessels were patent.

The liver weighed 1150 gm., was soft, and had a mottled blue, yellow, and white surface. The parenchyma displayed almost complete loss of normal liver architecture, with wedges of soft, homogeneous purple-red tissue among large yellow areas. A few intrahepatic vessels were surrounded by a thin dark-red rim. The spleen was soft and weighed 75 gm. There was a small, retracted fibrotic scar on the diaphragmatic surface, and the remainder of the splenic tissue was dark blue-red, the follicular and trabecular architecture being obliterated.

Fig. 2 (Case 1).—Cross section of mural fibrin thrombosis of aorta. Elastica Van Gieson stain.

The left ventricle of the heart was slightly dilated and hypertrophied, but the cardiac chambers and endocardial structures were otherwise unremarkable. The ascending aorta had tiny linear intimal atheromata, without wrinkling, arranged transversely, becoming longitudinal in the arch. One pale intimal plaque overlay an atheromatous deposit. Just as the arch became the descending thoracic aorta, in the ventral midline was an 11.2 cm. long granular brownish-pink mass on the intima. On its superior surface was a slightly depressed whitish area. Its inferior edge was continuous with an elevated longitudinal ridge with a smooth surface, 2 cm. wide, extending on the ventral surface of the aortic channel in the midline for almost the entire length of the thoracic aorta. Section of the ridge grossly revealed that the structure was 0.3 cm. thick and consisted of a glossy, gelatinous, grayish layer 0.2 cm. thick covered by a rim of pink tissue continuous with the intima on both sides of the ridge. At the inferior edge of the ridge, a few centimeters above the diaphragm, was the origin of another brownish-pink, moderately firm granular mass 3 cm. wide, which extended inferiorly for 7 to 8 cm., was surrounded by a rim of thickened intima, and was continuous at its lower end with a small glossy polypoid excrescence.

Multiple cross sections through each mass show that each was incorporated into part of the intima and subintima and that their maximum thickness was 0.7 cm.

They were composed of four layers, all of which were not present in all sections. From the media to the luminal surface they were as follows:

(*A*) An interrupted layer of a bright yellow color with a red-brown rim at some places. This layer was absent at the two ends and in the center of the mass.

(*B*) A layer with a glossy, homogeneous gray appearance was found in all cross sections, with a maximum thickness of 0.25 cm.

(*C*) An interrupted firm, dense, white layer with a maximum thickness of 0.35 cm. This layer was absent at places and intermingled with the next layer in others.

(*D*) The most superficial layer, which was present in all sections and was continuous with the intima on both sides of the ridge, was generally pink with whitish areas intermixed at some points and, at places, irregular red patches, often at the very surface. At the latter areas, an overlying, adherent granular, red and gray mural thrombus was present.

The media of the aorta appeared unaffected below the lesion.

Caudad to the above mass, the intima was grossly unaffected for a few centimeters. Then a similar third mass with similar characteristics, 4 cm. in length and 2 to 3 cm. in width, was present. This last mass overlay the orifices of the celiac axis and superior mesenteric artery and extended to within 1 cm. of the orifices of the renal arteries. Gross sections of this third mass revealed the four layers noted above. At the ostium of the superior mesenteric artery, the mass consisted solely of reddish tissue and appeared to be a relatively recent thrombus.

There was no evidence of atherosclerosis or arteriosclerotic plaque formation anywhere in the aorta other than the fine streaking noted above and a few small yellow plaques without erosion or calcification just above the bifurcation of the aorta.

Iliac Arteries: In the left common iliac artery, there was a lesion of the anterior wall 1.5 cm. wide and 2.5 cm. long. It was delineated by a rim of thickened intima, at the center of which there was an elevation in the upper half and a depression in the lower half. The elevated area had a smooth bluish surface, 0.55 cm. thick from media to lumen, and was composed of the following layers (from media to lumen):

(*A*) Bright yellow layer

(*B*) Thin glossy layer

(*C*) Layer of whitish tissue intermingled with glossy tissue

(*D*) Well-demarcated layer of red-gray material

(*E*) Thin, slightly translucent homogeneous layer, 0.15 cm. thick, which was continuous with the intima on both sides of the lesion

The ovoid depressed area had an irregular, but glistening, surface except in its superior portion, where it was covered by fresh thrombotic material lying contiguous to Layer D above. Cross section of the depressed region revealed layers similar to A and B above, covered by a third fragmented layer of pink material. The remainder of the vessels of the major circulation had rare, isolated atheromata without erosion or calcification. The pulmonary arteries had discrete yellowish plaques at their major bifurcations, but no erosions or calcifications.

Microscopic Findings.—The organs supplied by the celiac axis and superior mesenteric artery were massively infarcted. The major aortic lesion consisted of a superficial antemortem thrombus overlying a mass of fibrin. Discrete fibrin strands were identifiable in the outermost portion of the mass but became a relatively homogeneous structure with a tendency toward metachromasia as the deeper portion was approached. The deepest portion showed some fibroblastic cellular organization arising from the im-

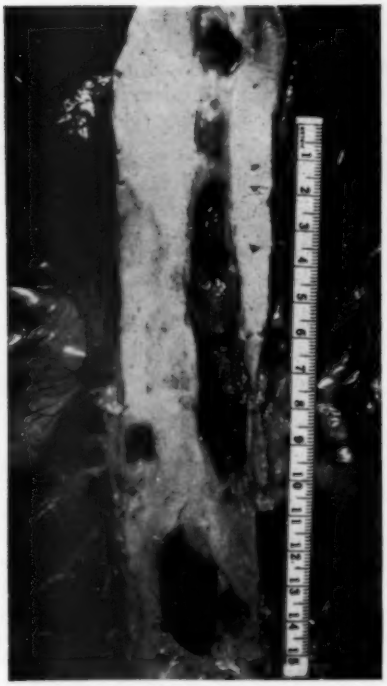


Fig. 4 (Case 2).—Mural fibrin thrombosis of aorta. This lesion is partially organized. The remaining aortic intima has a minimal degree of atherosclerosis.

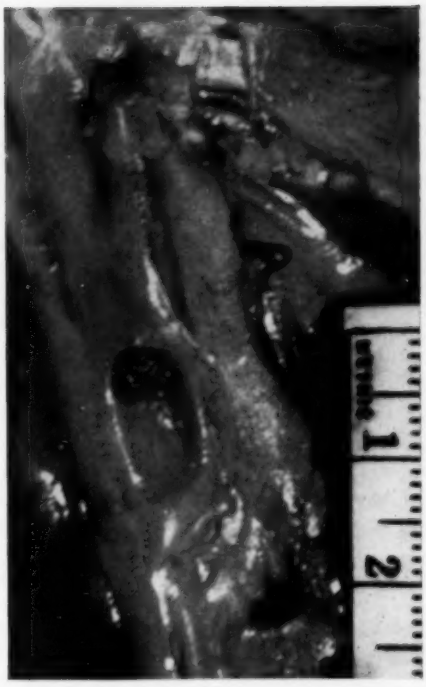
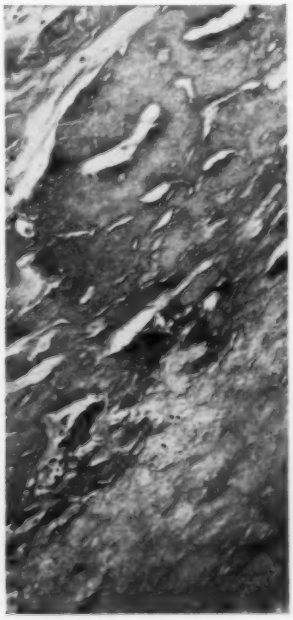


Fig. 5 (Case 2).—Fibrin thrombus in fusiform aneurysmal dilatation of the common iliac artery.

Fig. 6 (Case 2).—Juncture of fibrillar fibrin (above) and homogeneous fibrin (below) without organization. This area is from the center of the aortic thrombus.

Fig. 7 (Case 2).—Structure of the junction of the homogeneous fibrin (above) and the intima (below).



mediately underlying vascularized connective tissue. This thin layer of connective tissue blended indistinguishably with the subintima of the aortic wall. The entire structure rested on an aortic media which was vascularized by fine capillaries throughout its entire width. At some points along the length of the mass, there were flat aggregates of cholesterol crystals and finely divided fat in the subintima and resting against the media. At the lateral edges of the thrombus, the fibroblastic proliferation in the subintima rose in a cup-like fashion to encompass the limits of the thrombus. It is rapidly lost in the homogeneous fibrin layer. At rare points at the periphery of the thrombus, intimal cells extend over the homogeneous fibrin-like structure for a short distance.

COMMENT

The aortic mural thrombosis in Case 2 has been present for a considerable period of time, as proved by the extent of organization and vascularization of the aortic wall and base of the thrombus, as well as by the homogeneous transformation of the deeper portions of the thrombosed fibrin. The presence of the clearly demarcated layers of thrombotic material in various stages of degeneration and organization and the obvious recent caudad extension of the process suggest that the thrombus was propagated by repeated episodes of thrombosis on top of older thrombotic material. It is most significant that in all of the layers in which thrombotic material was identifiable this material was always acellular and appeared to be fibrin or a degenerating product thereof. The phosphotungstic acid hematoxylin stain always clearly defined the fresher fibrin strands at the luminal surface, the deeper homogeneous structure staining as "fibrinoid" material.

As in Case 1, no intrinsic disease at the aortic wall was found. Of course, the extent of the vascularization of the aortic wall underlying the thrombus confused the structure of the wall to a certain degree, but careful study does not reveal any underlying anatomical lesions which might have precipitated the initial thrombotic episode.

The iliac artery thrombosis, identical in structure with the aortic lesion, lay in a small fusiform aneurysmal dilatation of the vessel. This probably represents a site of predilection for the fibrin thrombus formation. The cause of the aneurysm was not anatomically definable, since no underlying alterations were found. No aneurysms were found in the aorta proper.

CASE 3 (MSH #15255).—*Clinical History.*—D. C., a 55-year-old white man with an eight-year history of hypertension reaching levels of 230/140, presented with progressive exertional dyspnea, orthopnea, and paroxysmal nocturnal dyspnea. He had a Grade III hypertensive retinopathy, cardiac enlargement with a blowing diastolic murmur in the fourth left intercostal space and a precordial systolic murmur. His serologic tests were negative. His urine had a low specific gravity, 3+ albuminuria, and occasional granular casts. The blood urea nitrogen ranged from 88 to 116 mg. per 100 ml. During hospitalization the patient had episodes of hemoptysis and progressive uremic signs and symptoms. Increased capillary permeability, skin petechiae, and vomiting were prominent, the latter producing a terminal hypochloremic acidosis. Finally, his urinary output decreased and a pericardial friction rub appeared. A gallop rhythm developed as he went into irreversible heart failure with marked drowsiness.

Gross Autopsy Findings.—The body was that of a 170 lb., well-developed but poorly nourished middle-aged white man. The significant gross pathology was in the cardiovascular system and kidneys. All chambers of the heart were moderately dilated, and both ventricles were somewhat hypertrophic (total heart weight: 550 gm.). The interventricular septum bulged into the right ventricular chamber. There was a considerable degree of yellow flecking of the myocardium and papillary muscles of the left ventricle and a minimal degree of diffuse myocardial fibrosis. Within the right auricular appendage was a typical adherent antemortem mixed thrombus. The coronary arterial system was markedly arteriosclerotic, with reduction of the lumina of all vessels and several old occlusions with gross evidence of recanalization by pinpoint-sized channels. The intima of the aorta was altered by numerous discrete, scattered plaques of atheromata and by calcified foci. Some of the latter were superficially eroded and ulcerated. The intervening intima was wrinkled. In the abdominal segment of the descending aorta were small pinkish-white masses adherent to the underlying aortic wall, the

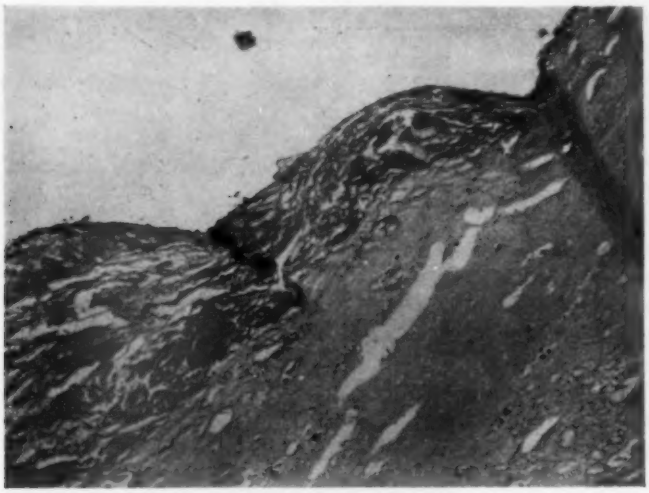


Fig. 8 (Case 2).—Edge of fibrin thrombus. Apparent extension of intima over the thrombotic material.

largest rising 1.0 cm. above the level of the adjacent intima. These masses were all of semispherical or ellipsoid shape. One of the masses lay over the orifice of the left renal artery, so that a probe could not be passed into the lumen of that vessel from the aortic side. The largest mass was attached to the aortic wall by a pedicle. Gross examination of the cross-sectional structure of the masses revealed four identifiable layers: (1) a base of thickened intima containing fat and calcified material, (2) a glossy white layer, (3) a thin red-pink line, and (4) the mass proper, which was composed of homogeneous white, firm but not hard, glistening material throughout which were a few minute gray strands and pinpoint red areas. The luminal surface of the mass was faintly pink. In a few areas, similar masses were present on top of more clearly defined atherosclerotic plaques. Within a single arteriosclerotic aneurysmal dilatation of the anterior wall of the abdominal aorta extending 5 cm. in cephalocaudad direction and 4 cm. transversely, similar masses to those described above were found. All the masses, both those in the aneurysm and those not associated with the dilatation, were present along the ventral midline intimal surface. A similar aneurysm of the right common iliac artery contained a similar mass. Both kidneys were the site of a severe degree of arterionephrosclerosis. The right kidney, in addition, had a 2.2×1.7×1.0 cm. pyramidal fibrotic scar.

Microscopic Findings.—Beside the severe atherosclerosis, scattered fibrosis, and fatty infiltration of the myocardium, and old renal

Fig. 9 (Case 2).—Vascularization of outer third of media.

infarction, the significant lesions were the masses on the aortic intimal surface. These masses all had the same basic structure, varying only in the thickness of the successive layers. The subintima of the aorta was thickened by fine fibers, fibroblastic proliferation, and extensive deposits of finely divided fat, cholesterol, and calcified material. The lipid and calcium deposits were beneath a layer of fibrous tissue containing vascular channels having the general architecture of granulation tissue. The upper extent of the granulation tissue layer was composed predominantly of numerous capillaries. This layer blended with the major portion of the mass. The inner two-thirds of the mass was composed of homogeneous basophilic to metachromatic material which stained as "fibrinoid" with phosphotungstic acid hematoxylin. Within this layer were scattered fibroblasts, more at the base and becoming rarer as the luminal surface was approached. The upper third of the mass was composed of interlacing strands of fibrin. A few of the masses, particularly from the aneurysmal area, had a further covering of mixed thrombus with formed elements as well as fibrin, but most were devoid of such a layer. The aortic wall underlying the thrombi was invaded by numerous capillaries and round cells. In the aneurysmal areas, occasional foci of cystic medial degeneration were found, in addition to the intimal atheromatous deposits. The identical thrombotic structure was found in the iliac artery.

Fig. 10 (Case 3).—Multiple mural thrombi in abdominal aorta. The lowest group of thrombi lie in an aneurysmal sac. The probe indicates the point of partial closure of the renal artery.

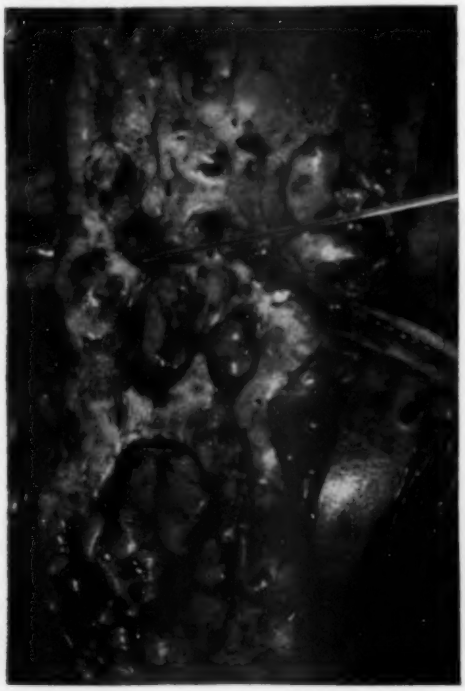


Fig. 11 (Case 3).—Aggregate of cholesterol crystals, calcium, and lipid in the base of the subintima. The thrombotic material is above, separated from the lipids by a thick layer of subintimal tissue.

COMMENT

Case 3 contains three aortic lesions, fibrin thrombi, arteriosclerotic aneurysmal dilatations of the anterior wall of the abdominal aorta and the common iliac artery, and severe arteriosclerosis. The first two appear to be related only to the degree that the aneurysms provided a preferential site of localization for the fibrin thrombi. This conclusion is supported by the observation that

fibrin thrombi undergoing extensive organization from the intima were present in the midline of the ventral surface of the aortic lumen in the nonaneurysmal portion of that vessel.

The thrombi projected into the lumen for a significant distance and were distributed so that the lateral edge of one of the thrombi caused partial occlusion of a renal artery. The hypertension, which formed a major clinical finding in this case, may have been invoked by a Goldblatt-type mechanism based upon partial occlusion of one renal artery. If such is the case, the responsible lesion must have been present at least eight years.

The presence of marked atherosclerosis under the fibrin masses is unlike the previously described cases. The relationship of these lesions suggests two possibilities: the first, that the fibrin deposited upon a preexistent plaque, and the second, that lipid deposition occurred after the thrombus formed. There is no means of deciding which sequence of events occurred by an isolated study of this case. However, if the degree of deposition and distribution of the lipids is compared with that seen in Case 2 and a similar comparison of the age of the thrombi is made, a suggestive pattern of development appears. The fibrin thrombi in Case 3 are older and more extensively organized than those in Case 2. Similarly, the lipid-cholesterol deposits are far more marked beneath the older lesion, although the distribution in the lower portion of the subintima is the same as in the fresher case. This evidence suggests that the lipid-cholesterol deposit followed the formation of the thrombus and increased with the increase in organization of the overlying thrombus.

GENERAL COMMENT

The cases presented illustrate the various stages of a clearly defined pathological entity. The original alteration is the formation of a fibrin thrombus on the anterior wall of the descending aorta. Although the masses of fibrin coagulum tend to form in relation to sites of aortic wall damage, the observation that they are also present on a portion of the aortic wall which is otherwise anatomically unaltered leads us to suspect that the basic change was not disease of the aortic wall. A striking feature of the lesion in all cases is the adherence of a large mass of fibrin to the vessel wall in the presence of a fast-moving stream of blood, and, at least in the first case, an elastic aortic wall. That the fibrin thrombus finally attained significant size and remained adherent to the intima under these conditions suggests that there may be a clotting mechanism alteration possibly based upon a defibrinating action in an agitated stream of blood. Unfortunately, the pathology in each of these cases was unrecognized during life, so that studies relative to the coagulation of blood were not available for analysis.

The cases under study clearly demonstrate the organization of the original fibrin thrombus from the underlying intima and subintima and the growth of intimal cells over the surface of the thrombus from the adjacent uninvolved intimal layer. The character of the organization is comparable to the organization of filiform fibrin thrombi in the formation of Lambl's excrescences described by Magarey.[5] During the phase of organization, further thrombosis may occur over the surface or at the caudal end of the primary fibrin mass. It is reasonable to assume that the secondary thrombosis appearing over the surface of the first thrombus is induced during the organizing period, when the luminal aspect of the mass offers a rough surface to permit the necessary fibrin deposition and cell entrapment of mural thrombus formation. The secondary thrombosis at the caudal end of the primary fibrin thrombus might be anticipated because the alterations in the luminal diameter at such a point of partial occlusion would set up abnormal flow patterns in the blood stream. This would occur most particularly at the point of sudden restoration of diameter, namely, at the caudal end of the thrombus. This would be responsible for the caudal progression of the thrombosis. The clinical syndromes

which may be expected are those due to obstruction to any tributary artery lying in the pathway of the progressively extending thrombus. Case 2 is an example of just such a sequence of events.

Thrombosis occurring in the descending aorta offers a site of origin for embolization to distal arteries. Whereas the vast majority of emboli originate in the chambers of the heart, from the venous circulation by "paradoxical embolization," or from the branches of the pulmonary veins, the lesions presented above offer an additional potential source of embolization. In Case 1 the saddle embolus was derived from a mural fibrin thrombus on the aortic intima. A diligent examination failed to reveal evidence of thrombosis in any of the more usual sites.

Dissection and microscopic study fail to explain the observation that the thromboses tend to form along the ventral wall of the descending aorta. It is possible that there are changes in the blood flow pattern at the end of the arch of the aorta which may be of significance in Case 1, but this would not explain the location of the lesions lower in the descending aorta. Alterations of total luminal diameter and distortion of the normal tubular structure of the aorta are of importance in Case 3 and, to a minor degree, in Case 2, but even here thrombi were found at sites that could not be explained on this basis. The possibility remains that an intrinsic disease of the aortic wall does exist, although it could not be demonstrated anatomically.

The iliac artery aneurysms found in Cases 2 and 3 and the aortic aneurysm seen in Case 3 do not appear to be part of the basic disease pattern. Thrombi were found in both cases on portions of the aortic wall uninvolved by the aneurysms. Careful search throughout the vascular tree failed to reveal thrombi of any type in other vessels or the microaneurysms of thrombotic thrombocytopenic purpura.

The designation of mural fibrin thrombosis of the descending aorta as a distinct pathological entity we believe to be fully justified. A summary of the findings in the three cases reported is presented in the Table.

Significant Findings and Criteria

	Case 1	Case 2	Case 3
Location of primary thrombus	Descending thoracic aorta	Descending thoracic aorta, common iliac artery	Abdominal aorta and common iliac artery
Distribution of primary thrombus	Ventral, midline	Ventral, midline	Ventral, midline
Method of propagation	Embolization	Contiguous propagation	Probably none
Significant arterial obstruction	Saddle embolus	Celiac axis, superior mesenteric artery	Left renal artery
Relationship to extravascular structures	None	None	None
Age of primary thrombus	Recent	Organizing	Organizing
Degree of subthrombotic lipid deposition	None	Slight	Severe
Anatomical factors predisposing toward localization	None	Aneurysm of common iliac artery	Aneurysms
Lesions of other vessels	None	None	Extensive arteriosclerosis

The clinical status of the patient and the progression of his disease does not seem to depend upon that unexplained mechanism which permitted the mural fibrin thromboses to form. Rather, the clinical syndrome is one of progressive arterial insufficiency of that area whose blood supply is compromised by the progression of the thrombus or by embolization from the primary thrombotic mass.

It is our opinion that the cases reported here do not confirm the thesis that typical atherosclerosis may be initiated by deposition of fibrin thrombi on the intimal surface of blood vessels. On the contrary, we believe

that mural fibrin thrombosis can be identified in any of its stages of progressive organization as an entity apart from atherosclerosis. An explanation must be sought for the appearance of lipid and cholesterol beneath the organizing thrombi, which probably occurs after the fibrin deposition. There must be a marked change in the physiological state of the subintimal tissues after a thrombus forms on the surface. Any transintimal perfusion of fluids must be altered, and significant changes in the chemical and physical state of the tissues can be expected. It is probable that the appearance of lipid in these tissues is associated with these changes.

SUMMARY

Three cases of mural fibrin thrombosis of the descending aorta are described. Most of the thrombi were localized in the midline of the ventral surface of the aortic intima. Underlying disease of the aortic wall offers sites of predilection for formation of the thrombi, but thrombi are also formed on anatomically unaltered portions of the aorta.

The possible mechanisms of this disease are discussed, as are the clinical and anatomical sequelae.

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Studies on Pancreatitis

III. Fulminating Hemorrhagic Pancreatic Necrosis Produced by Means of Staphylococcal Toxin

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The outstanding lesion of acute hemorrhagic pancreatitis is a massive and indiscriminate coagulation necrosis of parenchymal and supporting structures. While, on the one hand, a reasonable facsimile of the naturally occurring disease may easily be produced experimentally by any one of several methods,* yet the fundamental mechanisms causing the necrosis have not been elucidated.

The present work reports a hitherto undescribed method for producing regularly a most fulminating hemorrhagic pancreatic necrosis, using dilute staphylococcal toxin. It will also be shown that the pancreatic necrosis so produced is the result of prolonged and profound vascular spasm produced in the small interstitial blood vessels by the toxin. The implications of these findings in relation to the human disease will be discussed.

MATERIALS AND METHODS

Animals.—Adult rabbits, weighing 2 to 4 kg., of both sexes and mixed breeds, were used; also mongrel dogs weighing 8 to 12 kg.

Toxin.—The staphylococcal toxin used throughout these experiments was prepared from the Wood 46 strain. The preparation and standardization of this toxin have been described previously.[4] Initial supplies of the toxin were provided by Dr. H. D. Piersma, Lederle Laboratories, Pearl River, N. Y.

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From the Department of Surgery, University of Minnesota Medical School.

* References 1 through 3.

Antitoxin.—The antitoxin used was of equine origin and contained 800 units per cubic centimeter.

Injection Apparatus.—The injection apparatus consisted of a 2-liter bottle connected to a small graduated fluid reservoir, a mercury manometer, and a rubber inflating bulb. With use of this machine, small volumes of fluid could be delivered at constant pressure.

Intraductal Injection.—In the rabbit, the method was that previously described.[5] In each instance the duct was ligated after injection. In the dog, injections were made by inserting a blunt cannula into the inferiorly located duct of Santorini after ligation and division of Wirsung's duct.

Transillumination System.—This method has been described previously.[4]

Preparation and Staining of Sections.—Blocks of tissue were fixed in formol-saline solution and stained chiefly by the hematoxylin and eosin method.

CLINICAL AND AUTOPSY FINDINGS IN ANIMALS SURVIVING LONGER THAN SIX HOURS

In a brief preliminary study it became apparent that hemorrhagic necrosis could be produced by dilutions of toxin up to 1 : 100. Massive and rapidly fatal necrosis could be produced regularly by 1 : 10 dilution, and a less rapidly fatal form by 1 : 40 to 1 : 80 dilutions. Accordingly, 1 : 10 and 1 : 40 saline dilutions of toxin were used in all experiments. Intraductal injections were made by the standard method, the volume of fluid being 5 cc. and the injection pressure being 20 mm. Hg. The results of this study are summarized in Table 1, from which it will be seen that all animals receiving 1 : 10 dilution of toxin died within 24 hours. In this group a characteristic clinical course uniformly followed the toxin injection. The animals would recover rapidly from anesthesia and appear normally active for the first two to three hours; thereafter they would in-

Fig. 1.—Acute hemorrhagic pancreatic necrosis in dog dying 48 hours after intraductal injection of 5 cc. of 1:40 staphylococcal toxin at a pressure of 20 mm. Hg. There was considerable fat necrosis and 400 cc. of sanguineous fluid in the peritoneal cavity. Note the hemorrhage into the adjacent duodenum.

variably lapse into a shock-like state of depression. This would become progressively deeper until the animal died, generally 12 to 18 hours after injection. Direct observations of the pancreas at laparotomy showed a regular sequence of events. Within a few minutes of the start of the injection, clear fluid began to separate the pancreatic lobules in a manner identical to that seen when isotonic saline is injected intraductally. However, unlike the edema following saline injection, which is rapidly resorbed, the clear interlobular fluid increased considerably in volume during the first 45 minutes after injection. At the end of this period the gland was markedly edematous and indurated and the interstitial fluid, previously crystal clear, had by this time become opalescent. Incision into the edematous areas revealed that the fluid had coagulated. After this period there was no further increase in the size of the gland. After two hours numerous punctate purplish areas

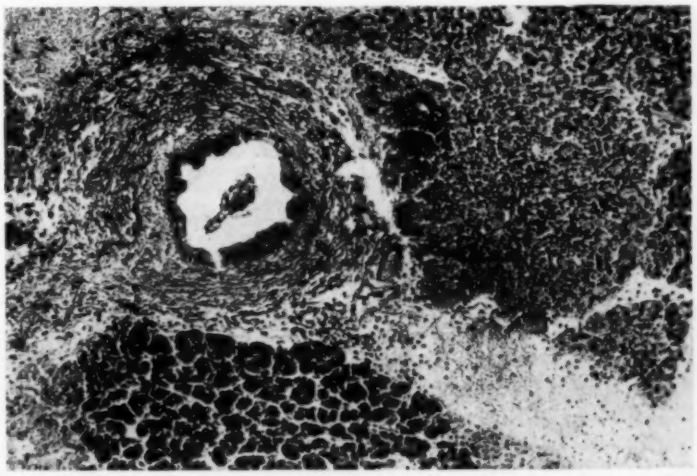

Fig. 2.—Early pancreatic necrosis in a dog which had received 5 cc. of 1:40 toxin intraductally 12 hours previously.

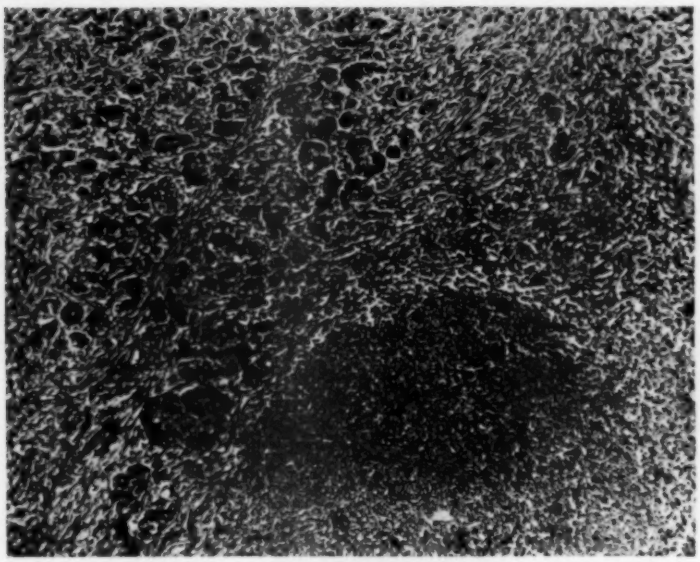

Fig. 3.—Healing phase at 8 days. This animal received 5 cc. of 1 : 40 toxin and was in good health when killed at 8 days. Section showing a few surviving acini in matrix of proliferating fibroblasts. In the lower part of the section an amorphous area of coagulation necrosis is seen.

were evident throughout the pancreatic parenchyma, and incision into the parenchyma at this time revealed only slight oozing of extremely dark blood.

The autopsy findings were strikingly uniform. Within the peritoneal cavity there was usually 250 to 800 cc. of serosanguineous fluid. Fat necrosis was extensive in those animals surviving longer than 12 hours and slight in those dying at an earlier stage. The greater portion of the pancreas was invariably transformed into a swollen indurated hemorrhagic mass.

In animals receiving 1 : 40 toxin, the onset of the shock-like picture was delayed until about 12 hours, and, though the animals were depressed, they were not prostrate. Vomiting was frequent in this group, and intravenous Ringer's solution was used to counteract the dehydration. As is shown in Table 1, seven of the eleven animals in this group died within 48 hours. All of these

TABLE 1.—*Acute Hemorrhagic Pancreatitis Produced with Staphylococcal Toxin* *

No. of Dogs	Toxin Dilution	Survival Time †	Pancreas	Fat Necrosis	Peritoneal Fluid
4	1 : 10 S-T	D 4-8 hr.	Massive hemorrhage and swelling of whole pancreas	Minimal	200-600 cc. serosanguineous
16	1 : 10 S-T	D 8-24 hr.	Massive hemorrhage and swelling of whole pancreas	Moderate	200-600 cc. serosanguineous
5	1 : 40 S-T	D 8-24 hr.	Massive hemorrhage and swelling of whole pancreas	Moderate	200-400 cc. serosanguineous
4	1 : 40 S-T	D 24-72 hr.	Large areas of hemorrhage and frank necrosis	Extensive	50-200 cc. straw-colored
4	1 : 40 S-T	K 6 days in good health	About ⅓ of pancreas hemorrhagic and necrotic; remainder indurated	Extensive	Minimal straw-colored
4	1 : 10 Toxin-antitoxin mixture	K 6 days in good health	Completely normal appearance	None	Minimal
4	1 : 10 S-T inactivated by heat	K 6 days in good health	Completely normal appearance	None	Minimal

* All intraductal injections were made at a pressure of 20 mm. Hg.
† D indicates died; K, killed.

showed large volumes of ascitic fluid at autopsy. The pancreas, though showing extensive necrosis and hemorrhage, was not completely destroyed, as it was in the group receiving 1 : 10 toxin. Areas of fat necrosis, both large and small, studded the peritoneal cavity. An interesting additional finding in two of these animals was segmental gangrene of areas of the duodenum abutting on the necrotic pancreas. Also, in several animals, there was marked hyperemia and a velvety thickening of the duodenal mucosa. The survivors also ran a characteristic course. For the first two days they appeared markedly depressed and ill, vomiting frequently. Thereafter, they improved rapidly, and when killed, on the eighth day, they appeared clinically well. It was consequently a surprise to find that all had extensive hemorrhagic pancreatitis, no less, in fact, than that found in those dying earlier. The pancreas in these animals was frequently hidden behind adherent loops of bowel and omentum and occasionally showed liquefaction in some areas, though the greater part was indurated and showed early organization microscopically.

To control the mechanical factor of ductal injection, four animals received toxin neutralized with specific antitoxin. This mixture exhibited neither hemolytic nor dermonecrotic properties. As will be seen in Table 1, these animals showed neither clinical nor morphological evidence of pancreatitis, though transient interstitial edema followed immediately on injection. Similar results were obtained when heat-inactivated toxin was used.

MORPHOLOGICAL ASPECTS OF THE LESION

At one hour the striking feature was the separation of the pancreatic lobules by fluid rich in fibrin. In general, the capillaries and veins were engorged with red cells and the arteries empty. In some areas there was diapedesis of red cells through capillary walls. The acinar cells were generally well preserved, though some showed large and small cytoplasmic vacuoles.

By three and one-half hours there were focal areas of interstitial hemorrhage. The source of the bleeding could frequently be identified as coming from the disrupted walls of small venules. Many venules showed swelling and hyalinization of their walls which were occasionally infiltrated by polymorphonuclear leucocytes, but more frequently by red cells. In general, the acinar tissue was fairly well preserved at this stage, though in some areas the cytoplasm was vacuolate, somewhat eosinophilic, and the nuclei were frequently pyknotic.

By eight hours there was indiscriminate and complete coagulation of all tissues in large areas. Thus, arteries, veins, ducts, and acinar tissue were represented by eosinophilic ghosts. By 18 hours there was extensive polymorphonuclear leucocyte reaction at the margin of the destroyed tissue in some animals. In general, there was intense venous and capillary engorgement. In many areas the veins were engorged with red cells which appeared agglutinated and altered in their staining reaction in such a way as to suggest that the blood in the channels was stagnant. Thrombi were uniformly absent during the first 24 hours and were rarely seen even at later stages.

By two and one-half days the area of coagulation was reduced to a hyalinized mass in which no remnant of the previous architecture could be detected. By four days there was an extensive infiltration by polymorphonuclear leucocytes and proliferation of macrophages in the necrotic areas, with marked proliferation of young fibroblasts in the periphery. There were also fairly large areas of what appeared to be recent hemorrhage. In the young scar tissue there were frequently found islands of surviving acini. By seven days the scar tissue had contracted to form broad dense fibrous septa, and in these areas the picture resembled that of an inactive lesion going on to fibrosis. Lesions of the larger arteries consisted mainly of eosinophilic necrosis of the muscular wall involving the whole or part of the circumference, as described by Rich and Duff.[6]

Occasionally in lesions older than 48 hours a frank arteritis with invasion of the arterial wall by polymorphonuclear leucocytes was seen.

MECHANISM OF PANCREATIC NECROSIS PRODUCED WITH STAPHYLOCOCCAL TOXIN

In the light of a previous paper,[4] wherein it was shown that the principal necrotizing property of staphylococcal toxin resides in its ability to produce local circulatory stasis, it seemed profitable to study this factor in the pathogenesis of the experimental disease. The following experiments were designed to study pancreatic blood vessel behavior and blood flow during the early development of the lesion.

Circulatory Stasis as a Prime Factor

With use of the transilluminated preparation previously described,[4] it was possible to study the intimate behavior of the minute lobular vessels in the living rabbit pancreas.

With the animal under pentobarbital (Nembutal) anesthesia, a midline laparotomy incision was made and the duodenal loop carefully delivered through the incision. An olive-tipped cannula was then inserted into the ampulla of the pancreatic duct and tied into place. The pancreas was then arranged on the Lucite reflector and the vessels and pancreatic lobules in the field carefully studied. Then 0.5 cc. of a 1:10 saline dilution of the toxin was injected at a pressure of 20 mm. Hg, using the injection apparatus already described.

As the toxin was injected, the ducts and ductules were seen to become slightly more prominent, and within a few minutes the capillary circulation within the pancreatic lobules became noticeably sluggish and soon ceased in several areas, the capillary vessels appearing segmentally engorged with packed red cells. At the same time, the veins began to develop areas of segmental spasm which, in the majority of instances, was so severe as to obstruct blood flow completely. The arteries also developed areas of severe spasm, but in general the venous and capillary changes preceded this by several minutes, accounting for areas where the pancreas was obviously engorged with stagnant dark blood. By 20 minutes circulation in the greater part of the pancreas had completely ceased and the normal pearl-gray pancreatic parenchyma had an engorged purplish hue. By one hour focal petechial hemorrhages, originating from ruptured capillaries, were seen throughout the gland. The venous and arterial spasm persisted throughout the period of observation, which varied from animal to animal but in general extended for about two to three hours. A further observation of interest was that in the remote areas of the pancreas where the toxin had failed to penetrate the lobules appeared entirely normal and the local circulation brisk.

In effect, the vascular changes preceding the pancreatic necrosis were in every respect identical to those previously demonstrated in the skin and mesentery.[4] A point of considerable interest was the apparent permeability of the duct system to the toxin. It thus became necessary to determine whether the toxin had diffused through intact duct walls or had in some way brought about their disruption.

Integrity of the Duct Wall

To find out whether the duct walls had undergone dissolution by the toxin, the following experiment was carried out:

Four rabbits underwent the standard injection of 0.5 cc. of 1:10 staphylococcal toxin. The cannula was left in place. At various intervals after the toxin injection, India ink diluted 1:20 in 0.9% NaCl was injected at the standard pressure of 20 mm. Hg. The ink outlined the minute ducts throughout the major part of the gland, but in no case could it be demonstrated either grossly or microscopically within the interstitial tissues, showing that there was no breaching of the duct wall. The pressure was then progressively increased until duct rupture occurred.

These results are summarized in Table 2. It will be seen that duct-rupturing pressure

TABLE 2.—*Effects of Staphylococcal Toxin on Duct-Rupturing Pressure of Pancreatic Ducts in the Rabbit*

Rabbit No.	Interval Between Injection of Staphylococcal Toxin and Attempted Duct Rupture	Duct-Rupturing Pressure, Mm. Hg
722	Saline control 30 min.	250
728	Saline control 1 hr.	250
729	Saline control 1 hr.	300
730	Saline control 2 hr.	200
731	Saline control 4 hr.	250
732	30 min.	200
733	1 hr.	200
734	2 hr.	250
735	4 hr.	250

did not differ significantly in the group of animals previously injected with toxin and the control animals, which received only saline.

SUPPRESSION OF PANCREATIC BLOOD FLOW DURING THE DEVELOPMENT OF PANCREATIC NECROSIS IN DOGS

The density and thickness of the pancreas in dogs makes transillumination impractical. To study alterations in pancreatic blood flow during the early development of pancreatitis the intra-arterial dye method was used.

Six dogs were given the standard intraductal injection of 5 cc. of 1:10 saline dilution of staphylococcal toxin at 20 mm. Hg pressure during laparotomy. At intervals thereafter 50 cc. of 1:20 India ink was injected percutaneously into the midthoracic aorta, and the pancreas and abdominal viscera were closely observed. Only a single aortic injection was made in each dog. The abdominal aorta was clamped below the renal vessels to increase the dye concentration in the abdominal viscera.

The observations are summarized in Table 3. It will be observed that the pancreas and abdominal viscera rapidly assumed a uniformly coal-black color in injections made in the control animals and those in which the toxin had been injected less than 20 minutes previously. In animals which had received toxin injections one or two hours previously the major part of the pancreas, which by this time was markedly edematous and indurated, consistently failed to blacken.

Remote areas of the pancreas which the toxin had failed to reach showed neither edema nor hemorrhage, and after India ink injection these areas took on a black color contrasting sharply with the mottled white and red appearance of adjacent areas which had been exposed to toxin. Furthermore, in animals allowed to survive it was found that hemorrhage and necrosis were confined to areas previously shown to be ischemic.

It would seem apparent from these observations that the areas of the pancreas destined to become necrotic undergo a profound diminution in blood flow soon after the toxin gains access to the interstitial blood vessels. Moreover, this prolonged cessation of pancreatic circulation would appear to be the

TABLE 3.—*Suppression of Pancreatic Blood Flow During Development of Pancreatic Necrosis in Dogs as Studied by Intra-Arterial Dye Injection*

Dog No.	Interval Between Toxin and Dye Injection	Results of Dye Injection	
		Pancreas	Other Abdominal Viscera
1	10 min.	Black	Uniformly coal-black
2	10 min.	Black	Uniformly coal-black
3	1 hr.	White, edematous	Uniformly coal-black
4	1 hr.	White, edematous	Uniformly coal-black
5	2 hr.	White, edematous	Uniformly coal-black
6	2 hr.	White, edematous	Uniformly coal-black
7	Saline control 30 min.	Coal-black	Coal-black
8	Saline control 30 min.	Coal-black	Coal-black
9	Saline control 2 hr.	Coal-black	Coal-black
10	Saline control 2 hr.	Coal-black	Coal-black

prime cause of the extensive coagulation necrosis which so prominently characterizes the experimental lesion.

COMMENT

The fulminating gangrenous pancreatic necrosis which regularly follows injection of dilute staphylococcal toxin into the pancreatic duct provides an interesting model of the human disease.

Unlike other methods used in the production of experimental pancreatitis, the action of the toxin is not dependent upon high injection pressures and ductal rupture.[7] Indeed, the pancreatic necrosis is just as severe when this substance is injected at pressures of 15 mm. Hg as it is at 300 mm. Hg. As demonstrated by Rich and Duff,[6] the pancreatic duct wall is readily permeable to saline and, as is shown in the present experiments and those reported in a previous paper,[5] it is similarly permeable to saline dilutions of certain bacterial toxins.

Direct observations of the living rabbit pancreas show that the initial change following the development of the pancreatic edema is a striking and rapidly developing venous spasm and capillary stasis. There is also associated arterial spasm. The engorgement of the capillaries and venules, however, makes it highly probable that venous spasm precedes arterial spasm. The resulting prolonged stagnation of pancreatic circulation would appear to be a most important factor in the subsequent parenchymal coagulation. This reaction is further substantiated by previous studies on the effect of staphylococcal toxin in the skin,[4] where it was shown that the prolonged local ischemia was of such duration as to cause dermal necrosis. It would seem that a similar mechanism is operative in this form of experimental pancreatitis. Further evidence on this score is provided by the studies on dogs, where it was shown that the areas of the pancreas exhibiting induration and edema were totally excluded from blood flow, whereas those areas to which the toxin had obviously not penetrated maintained their normal appearance and normal blood flow. Subsequent studies substantiated the conclusion that the areas deprived of circulation underwent massive pancreatic necrosis.

While it must be conceded that these observations apply only to this particular form of experimental pancreatitis, yet the implication may be derived from this work that local cessation of pancreatic circulation may bring about rapid dissolution of the gland. The idea that hemorrhagic pancreatic necrosis results from local disturbances in blood flow is not new and was first proposed by Panum [8] in 1862, who produced focal areas of hemorrhagic necrosis by injecting wax particles into the pancreatic arteries. Similar experiments have been performed by Lepine,[9] Bunge,[10] Smyth,[11] and, most recently, MacKenzie.[19] Indeed, the concept of a vascular cause for this disease must have occurred to the first workers who observed the infarct-like coagulation necrosis and the rare but well-documented occurrences of classical hemorrhagic pancreatic necrosis in cases of embolism to branches of the celiac axis.† In spite of these early observations, it is only in recent years that attention is once more being directed to the role of vascular factors in this disease.‡

The hiatus in the vascular theory resides in the provision of a mechanism whereby primary cessation of pancreatic blood flow might be produced, for obviously embolic pancreatitis or primary splenic vein thrombosis could be but exceptionally rare causes of the disease. Opie's original observation of hyalin capillary and venular thrombosis,[1] later confirmed by others in autopsy material,§ provides a mechanism whereby extensive interference with pancreatic circulation might be brought about. Experimentally, it has been shown by means of the local Shwartzman reaction [5] and also the Arthus reaction [15] that primary thrombosis of capillaries and venules results in classical hemorrhagic pancreatic necrosis. The present work demonstrates that the disease may be

† References 11, 18, and 19.

‡ References 11, 12, and 13.

§ References 11 and 14.

produced experimentally by a primary vasoconstriction, in this case induced by staphylococcal toxin.

Whether or not bacteria or bacterial products do play a part in the pathogenesis of pancreatitis is at present unknown, as adequate bacteriological studies of this disease are comparatively rare. It suffices to say that in the relatively few instances where bacteriological cultures were made a significant proportion were positive. Thus, Schmieden and Sebening [16] report 103 positive cultures of peritoneal fluid in pancreatitis and 84 negative cultures, 54 positive cultures of the bile and 40 negative cultures. The organisms found were Escherichia coli, staphylococci, and streptococci. Koller [17] describes the occurrence of fatal interstitial pancreatitis provoked by ingestion of codfish infected with hemolytic streptococci. In these two cases, the organisms were cultured from the pancreas' peritoneal effusion and demonstrated within the pancreatic interstitial tissue histologically. Moreover, in a recent study of 31 fatal cases of hemorrhagic pancreatitis, bacteria were demonstrated histologically in the gland in six cases.[14] While not in any sense proving that bacteria play a role of any great importance in the pathogenesis of pancreatitis, these observations do suggest that this aspect of the problem requires further study. The mere presence of microorganisms in the duct is probably insufficient cause per se to precipitate the disease.[6] It would seem that several factors in combination are required, of which the vascular effects of the particular organism or its products and the factor of ductal obstruction may well be the most important.

While the pancreatic necrosis produced by staphylococcal toxin illustrates only a purely experimental method of reproducing the disease, it has implications in the broader sense, for it demonstrates that this most fulminating form of experimental pancreatic necrosis is in all probability the result of suppression of blood flow. If this is true of the naturally occurring disease, it would serve to emphasize not only the important role which bacteria or bacterial products may play in producing local ischemia but also that any process interfering with pancreatic blood flow, whether by traumatic, infectious, or chemical means, may precipitate the massive destruction which characterizes the human disease.

SUMMARY

Severe and highly fatal hemorrhagic pancreatic necrosis was regularly produced in dogs and rabbits by introducing staphylococcal toxin into the pancreatic duct at low pressures.

It was found that staphylococcal toxin diffuses through the duct wall readily without producing an anatomical breach. Heat-inactivated and antitoxin-neutralized toxin were quite without effect in producing pancreatic necrosis.

The underlying cause of the extensive pancreatic necrosis was shown to result from suppression of pancreatic circulation induced by the toxin.

The significance of this finding and the role of bacteria and bacterial products in the pathogenesis of the human disease is briefly discussed.

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Histochemical Studies of Fibrinoid Substances and Other Abnormal Tissue Proteins

IV. Protein Character of Amyloid

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Investigations of the fundamental nature of amyloid and the mechanisms concerned with its formation have been limited. A variety of staining procedures have demonstrated the metachromasy of amyloid and its ability to color Schiff's reagent after periodic acid oxidation.[22] Hass and his associates * have analyzed amyloid and noted that it is a protein-mucopolysaccharide complex. The polysaccharide moiety contained sulfur, nitrogen, hexosamines, and uronic acid. A protein component was present but its identity could not be determined. Most workers agree that the protein constituent of amyloid has not been characterized with any degree of certainty.[13] Hass and his group presented evidence that the polysaccharide constituent represented a sulfated polymer similar to chondroitin sulfate.

The purpose of this paper is to present the results and possible significance of an analysis of human amyloid. Filter paper electrophoresis and microchemistry methods were utilized.

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* Reference 5 and 6.

MATERIAL

Liver was obtained at necropsy from two cases of amyloidosis. Patient A, a 51-year-old white man (A15823), was clinically an unsuspected case, while Patient B (A16301) was diagnosed during life by a liver biopsy. Patient B was a 38-year-old white woman, who was admitted with a classic nephrotic syndrome. In both cases there was no evidence of multiple myeloma or of a chronic infection. The liver weights of A and B were 3650 and 3400 gm., respectively. Gross examination revealed similar findings in the two cases. Color was dull gray-brown with streaks sharply delineated against the lusterless surface. The organs were extremely firm, and on cut section the surface revealed almost a total disappearance of hepatic parenchyma. Lobular architecture was completely destroyed, and remnants of parenchyma appeared as brown arborizing streaks. The intrahepatic vascular and biliary channels were patent, but the lumina seemed to be reduced in caliber. In addition to the liver, Case A revealed the spleen, kidneys, adrenals, gastrointestinal tract, and meninges to be involved. The spleen and kidneys were the only other organs grossly involved in Case B. Both cases may be regarded according to accepted classifications as primary amyloidosis, although the organ distribution was that commonly observed in secondary amyloidosis.

Tissue from Patient A was obtained six hours after death and immediately processed. Although the autopsy of Patient B was performed 4 hours after death, refrigerated tissue (20 C) was not available for analysis for an additional 48 hours. The liver was easily sliced into thin strips and repeatedly washed in ice-cold isotonic saline. Tissues were processed in batches of 100 gm. (wet), and a total of 300 gm. was utilized in each case. Tissue was homogenized in ice-cold 0.25 M sucrose solution in an all-glass homogenizer at 4 C. The homogenate was filtered through rough gauze in order to separate coarse fibrous elements. A simple modification of Hogeboom's method of differential centrifugation at relatively low speed allowed the separation of two major fractions. Fractions IA and IB, or the supernate, contained liver cells, nuclei, and debris. Fractions IIA and IIB, the sediment, were

smeared and stained with toluidine blue, thionine, and methyl violet. Fraction IIA stained intensely red with methyl violet, while metachromasia was less pronounced with the other dyes. This fraction appeared to be almost pure amyloid and was opaque, gray-pink in color. Fraction IIB yielded somewhat similar staining reactions but much more reduced in intensity. The smear preparation of IIB was not as homogeneous as IIA and contained more nonmetachromatic material. This component was a muddy red when stained with hematoxylin and eosin. No further identification of this substance was carried out at this time.

Control tissues consisted of 100 gm. (wet) of liver from postmortem cases within six hours after death. The cases selected were obstructive jaundice due to carcinoma of the pancreas, Laennec's cirrhosis, cardiac hepatic fibrosis, and myasthenia gravis (normal liver). All tissues were obtained from necropsies performed in the Department of Pathology, Mount Sinai Hospital, numbers A15844, A15611, A15806, and A15652, respectively. These tissues were prepared in an identical manner and processed in accord with the procedures mentioned above.

When Fractions IIA and IIB were prepared, they constituted approximately 60% to 70% of the homogenate. Fraction II obtained from the control tissues usually represented 4% to 10% of the total sample. Smears of control Fraction II revealed an eosinophilic substance which was generally smooth but showed occasional eosinophilic short fibers. This material, when stained with methyl violet, toluidine blue, and thionine, showed a mild degree of metachromasia with toluidine blue only.

METHODS

Fraction II tissues (from the amyloid and control tissues) were extracted with an ether-ethanol mixture at room temperature and at 60 C. The resulting material was evaporated to dryness and then dissolved in barbital buffer pH 9.0. At this pH almost all of the amyloid material went into solution, except for a small amount which entered into solution only when 0.1N sodium hydroxide was added. The barbital preparation was used throughout for the electrophoretic studies. The protein patterns obtained with the sodium-hydroxide-dissolved amyloid residue were difficult to evaluate and are not included in this report. The control tissues were easily prepared in the barbital buffer.

Horizontal filter paper electrophoresis was performed according to the method of Kunkel and Tiselius.[9] Vertical separation was carried out by means of the Durrum type cell.[8] The volume of Fraction II added to the paper was 0.1 to 0.2 ml. as a transverse band approximately 1 cm. wide. Barbital buffer pH 8.8 was used in the cell, and a current of 10 to 20 ma. maintained from a power supply unit. Protein separations were allowed to run 18 hours, and then the paper strips were processed according to the method of Kunkel and Tiselius. Bromphenol blue demonstrated the protein fractions, and the dye was eluted from segments of the paper strips for spectrophotometric analysis at 575μ. Sera from a case of multiple myeloma and a normal adult man served as controls.

The method of Kunkel and Slater[10] was used to obtain lipoprotein patterns. Strips were cut up into ½-in. sections and placed in a 20-cc. hot alcohol-ether solution. The extraction procedure followed the original method. However, while these workers determined cholesterol, phospholipid, or total lipids, it was found more useful in the present study to confine the determinations to cholesterol alone. Since the cholesterol curve closely paralleled the phospholipid and the serum protein curves, cholesterol determinations roughly correlated with lipoprotein distribution in the protein fractions. The method of Swahn[24] was investigated but considered inconstant in its reproducibility. The demonstration of lipids after electrophoresis was successfully accomplished by immersing the strip in a saturated solution of Sudan IV in 40% ethanol, 50% glycerin, and 10% distilled water.[19] Paper strips remained in this solution overnight at room temperature, then were washed and dried. Areas rich in lipid stained dark red against a pale pink background.

In the present study, protein separation was carried out as usual with the Durrum apparatus, and the paper strips were then stained with 0.01% toluidine blue in 0.5% aqueous acetic acid. The dried papers were dipped into the staining bath, quickly withdrawn, and blotted. The acid mucopolysaccharides were determined quantitatively by a densitometric technique. A sample of chondroitin sulfate was found to stain as a distinct reddish band on a blue background. The periodic acid-Schiff technique proved to be unsatisfactory because of the instability of the color and inconstant staining of control samples.

A serum sample from Patient B (initials C. K.) was obtained 20 days before death and was analyzed for proteins, lipoproteins, and protein-bound carbohydrates by the same methods previously mentioned. Lipoproteins were determined by a recording densitometer of a Sudan IV or oil Red O stained paper strip. In addition, a serum globulin distribution pattern was determined by chemical methods. Serum from Patient A was not available for study.

RESULTS

The electrophoretic patterns of a normal serum (25-year-old white man) as contrasted with the abnormal proteins present

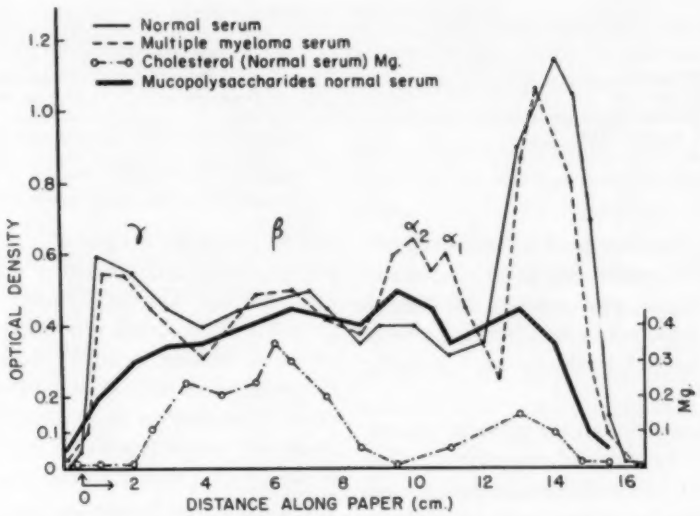

Fig. 1.—Electrophoresis of normal serum protein, lipoprotein, and mucopolysaccharide. Multiple myeloma serum with elevated α-globulins.

in the serum of a 70-year-old Negro man with multiple myeloma are demonstrated in Figure 1. A marked elevation of the α_1- and α_2-globulin fractions of the myeloma serum is noted. In the normal serum, the mucopolysaccharides present occur as sharp peaks in the albumin and α_2-globulin regions. The lipoproteins primarily migrated with the β-globulin fraction. Similar results were obtained when the paper strips were stained by the Sudan IV preparation and then scanned by a densitometer. Figure 2 demonstrates the serum electrophoretic patterns for protein, lipoprotein, and mucopolysaccharides in Case B. There is a decreased albumin and a significantly elevated β_1- and β_2-globulin zone. The apparent elevated lipoprotein in the gamma-globulin area is an artifact due to the nonspecific adsorption of dye at the point of serum application. This has been referred to as the 0 or origin staining zone

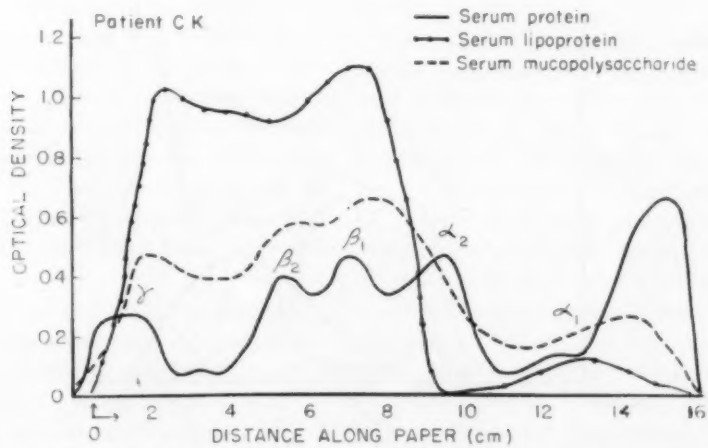

Fig. 2.—Electrophoresis of serum from Patient B (C. K.). Note elevated α_2- and β-globulins along with marked increase in β-lipoprotein. Mucopolysaccharide elevated in zone of β-globulins.

TABLE 1.—*Serum Globulin Pattern*

	Normal	C. K.
Protein-polysaccharide (PTP)	90-135 mg./100 cc.	149 mg./100 cc.
Mucoprotein (M)	40- 70 mg./100 cc.	45.8 mg./100 cc.
Acid-precipitable globulin turbidity (APG)	4-8 units	13.5 units
$ZnSO_4$ turbidity (ZS)	4-8 units	3.2 units
PTP/M	1.2-2.4	3.2
APG/ZS	0.6-1.8	4.2

by previous workers.† The mucopolysaccharide serum content begins to rise in the α_2-globulin region and reaches its highest value in the β-globulin zone, dropping slowly but not to normal levels in the γ-globulin region.

Table 1 shows the serum globulin pattern as determined by chemical procedures.‡ The distribution of polysaccharide bound to total protein (PTP) is uneven, with one-half in the α, one-fourth in the β, and one-fourth in the γ serum protein fractions. By this method albumin has no polysaccharide; however, by filter paper electrophoresis a polysaccharide component may be identified in the albumin fraction. The serum mucoprotein determination measures the α_1- globulin protein. Acid-precipitable globulin turbidity (APG) measures the α_2- and β-globulins. Serum γ-globulin is conveniently determined by the zinc sulfate turbidity method (ZS). Greenspan has utilized certain ratios as indices of alterations in globulin distribution. Thus, the values listed in Table 1 reveal an increased total bound polysaccharide, normal mucoprotein, increased α_2- and β-globulin, and decreased γ-globulin. According to Greenspan, the ratios indicate elevated α_2- and β-globulins. Except for the discrepancy between the chemical and the electrophoretic procedures regarding polysaccharide in the albumin fraction, there is a high degree of correlation of the globulin distribution patterns between the basically different methods. The assumption is made that the electro-

† References 19 and 24.

‡ Dr. Ezra Greenspan made the determinations.

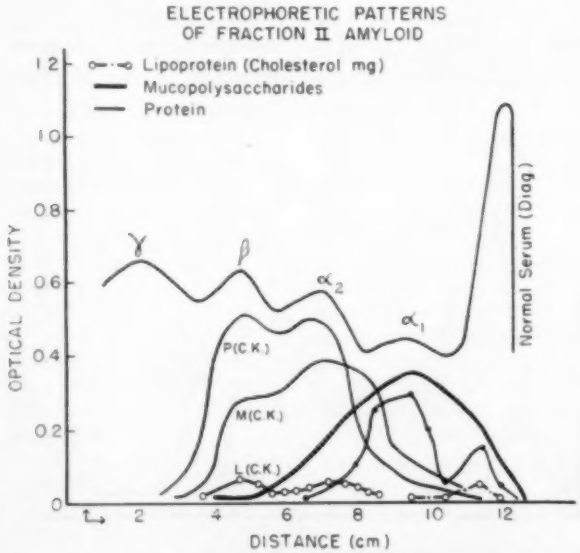

Fig. 3.—Electrophoretic patterns of amyloid compared with normal serum. Patient A shows prominent polysaccharide and protein component in α_1-globulin boundary. Patient B (C. K.) shows elevated polysaccharide-protein values in the α_2- and β-globulin zones.

phoretic determinations are more sensitive than the in vitro measurements, allowing for the detection of polysaccharide in trace amounts.

Figure 3 demonstrates the electrophoretic patterns of amyloid as Fraction II compared with the protein distribution of normal serum. Amyloid from Case A shows a protein component with a mobility similar to α_1-globulin and albumin. An extremely prominent mucopolysaccharide fraction is noted with a mobility of an α_1-globulin. The patterns obtained from Case B (C. K.) contrast sharply with those determined in Case A. The protein component shows two peaks corresponding to the α_2- and β-globulins. There is a distribution of the mucopolysaccharide component in the α_1-, α_2-, and β-globulin fractions. In both cases, A and B, lipoproteins were faintly detected. The electrophoretic studies were repeated at least five times for each component, and always with use of a normal serum control. The curves presented in Figure 3 represent the calculated average results obtained from these multiple electrophoretic runs.

Table 2 is a tabulation of the results obtained from filter paper electrophoresis of the control liver fractions compared to the two cases of amyloid. When repeat determination gave inconstant trace patterns, results were regarded as negative. Optical density measurements up to 0.05 were recorded as ± quantities. Fraction II from the liver of obstructive jaundice gave values of this type for α_1- and α_2- globulins. This same tissue sample showed a ± lipoprotein migrating in the β-globulin fraction. Values of ± were obtained for mucopolysaccharides in the α_1-globulin fraction from the cirrhotic and obstructive jaundice livers, while only the latter showed a similar value in the α_2-globulin region. The cirrhotic preparation yielded a 1+ (O. D. 0.15) value for mucopolysaccharide in the α_2-globulin fraction. It becomes obvious that the amyloid samples contain unusual concentrations of protein and polysaccharide, which are easily recognized by electrophoresis.

TABLE 2.—*Tissue Protein Patterns (Fraction II)*

Liver	Albumin	Globulin				Lipoprotein
		α_1	α_2	β	γ	
Normal	−	−	−	−	−	−
Obstructive jaundice	−	±	±	−	−	±
Cirrhosis	−	−	−	−	−	−
Fibrosis	±	−	−	−	−	−
Amyloid	+	+++	+	−	−	−
Amyloid (C. K.)	−	−	++++	++++	−	−
		Mucopolysaccharides				
Normal	−	−	−	−	−	..
Obstructive jaundice	−	±	±	−	−	..
Cirrhosis	−	±	+	−	−	..
Fibrosis	−	−	−	−	−	..
Amyloid	++	++++	++	−	−	..
Amyloid (C. K.)	−	++	++++	+++	−	..

Early in this study, the Kunkel-Tiselius cell for horizontal paper electrophoresis was used to advantage. As the number and scope of the determinations increased, the vertical technique was adopted for its ease of operation. Electrophoresis of duplicate samples for protein, lipoprotein, and polysaccharide containing protein were almost identical by the two methods.[23] The concentration of toluidine blue employed by Rienits,[17] 0.125%, was noted to favor dye adsorption by the paper, tending to obscure the boundaries of the polysaccharide. Toluidine blue reduced to a 0.01% concentration sharply delineated the metachromatic zones from the light blue background.

Highly polymerized hyaluronic acid and chondroitin sulfate § were easily identified after separation by electrophoresis. Depolymerized samples were more difficult to localize owing to increase in the intensity of the blue background. The PAS staining procedure showed no apparent relationship between the degree of polymerization and stain intensity. A somewhat similar observation has been made by Rienits.[17]

COMMENT

Since the work of Hass and his associates on the chemical composition of amyloid, a review of the literature fails to reveal a comparable study. Owing to the not infrequent association of multiple myeloma and amyloidosis, there have been numerous reports describing the serum protein aberrations and their causal relationship to amyloid.‖ The protein abnormalities are almost always of the globulins and, depending on which component is elevated, determine the serum designation. Reiner and Stern,[29] in a study of 91 myeloma patients, found 6.6% α, 15.4% β, and 56% γ patterns. The remaining 20% showed minor variations. Such a variation is the α_1, α_2 pattern in Figure 1.

The implications of such a nomenclature are that similarities in electrophoretic mobility of abnormal and normal serum proteins render them comparable. This concept forms the basis for interpretation of abnormal tissue proteins as submitted in this paper. These proteins may be further characterized by comparison with the mobilities of purified, isolated proteins. Such a method suffers from the inherent weakness of indirectness and only suggests physical identity. Chemical dissimilarity in the presence of physical similarity is indicated by immunologic studies demonstrating differences between myeloma and normal serum globulins.¶ Putnam has recently noted differences in the end-groups of myeloma globulins.#

Hoessly and Greenberg [7] studied a case of multiple myeloma with amyloidosis having an elevated serum β_1- and β_2-globulin. Sachs and co-workers [20] investigated the sera of 11 myeloma patients by paper electrophoresis determinations of the serum protein, lipoprotein, and carbohydrate patterns. An abnormal lipid was detected in seven cases having a mobility similar to the elevated globulin component. Carbohydrates, identified by the PAS method, were localized in the same areas as the abnormal lipid and globulin. These workers suggest that there is an abnormal protein-lipid-carbohydrate complex in the serum of certain myeloma patients. On the other hand, Lewis and Page [12] analyzed the lipoprotein patterns in 21 myeloma patients by the conventional Tiselius technique and ultracentrifugation. In this study, no correlation between the serum protein pattern and the lipoprotein pattern was observed. Sera with the greatest increase in total protein were noted usually to possess low concentrations of β-lipoprotein. Very low β_1- and α_1-lipoproteins were demonstrated in sera often having the largest globulin concentrations. The apparent conflicting data further emphasize the need for careful and extensive study of serum protein-lipid complexes in disease states. Trautman [26] has urged caution in the interpretation of electrophoretic results in the presence of lipoproteins, owing to nonplanar difficulties in the moving-boundary.

Rienits, studying the electrophoresis of acid mucopolysaccharides on filter paper, noted that while no single dye was entirely satisfactory in showing the position of these substances toluidine blue and azure A were the stains of choice. However, the work of Köiw [8] indicated that the periodic acid-Schiff stain could be applied to the paper strips with adequate demonstration of protein-bound carbohydrates. He observed that the carbohydrates appeared as sharply differentiated violet-red bands. By use of a photoelectric

§ Hyaluronic acid, umbilical cord, from Worthington Laboratories, N. J.; chondroitin sulfate, from Wilson Laboratories, Chicago.

‖ References 7, 13, and 22.

¶ References 21 and 22.

References 15 and 16.

colorimeter, the quantitative estimation of protein-bound carbohydrates was obtained by direct reading from the paper strip. Leitner and Kerby [11] studied the staining of acid mucopolysaccharides after chromatography on filter paper and obtained satisfactory results using an aqueous acid solution of toluidine blue.

Using paper electrophoresis, Rice [18] studied 12 cases of multiple myeloma. Ten of these cases showed an increase in PAS material corresponding to the mobility of the abnormal protein. In two cases PAS-positive material appeared to vary independently of the globulin. Rice suggests that perhaps the polysaccharide-containing protein circulating in the blood is related to the deposition of amyloid. A similar possibility is implied by Sachs, Cady, and Ross.[20] The studies of Rice and Sachs and co-workers concerning the distribution of the polysaccharide-containing protein in myeloma sera are in good agreement.

As indicated in Figure 2, the elevated serum polysaccharide, lipoprotein, and globulins move as a complex when studied by filter paper electrophoresis. This patient, although not suffering from multiple myeloma, revealed a serum pattern similar to those found in myelomatosis. That this patient also exhibited amyloidosis is of further interest, since there is a relatively high frequency of amyloidosis associated with multiple myeloma. The serum patterns obtained in this case are closely similar to the β-myeloma sera of Sachs and co-workers. Figure 3 shows that in Case A the amyloid was largely composed of α_1-globulin and mucopolysaccharide migrating as a complex. The amyloid from Case B appears to be composed of α_2- and β-globulins, with a corresponding mucopolysaccharide complex. The mucopolysaccharide in this case extends into the α_1-globulin region also. There is no significant amount of lipoprotein present in the amyloid studied. Detailed histochemical studies (to be published)[27] of the amyloid from these cases has failed to identify lipids. The histochemical observations confirm the presence of a protein-mucopolysaccharide complex in amyloid.

Thus, the combined serum-amyloid protein studies tend to suggest a relationship between the circulating abnormal complex and the protein composition of amyloid. The findings in Case B demonstrate this possible relationship in a dynamic manner, and such evidence is presented for the first time. The elevated lipoprotein values may merely reflect the nephrotic state and have no significance in the formation of amyloid. In both cases, the amyloid failed to reveal significant quantities of lipoprotein.

Hass has implied that the mucopolysaccharide of amyloid may be similar to chondroitin sulfate. Carnes and Forker [2] recently indicated that by dye absorption techniques the mucopolysaccharide component of amyloid differs from chondroitin sulfate. The electrophoretic studies presented in this paper do not clarify this problem. However, histochemical analysis of the amyloid obtained from Cases A and B confirm the impression that the mucopolysaccharide moiety is not chondroitin sulfate.[27] The exact nature of this complex compound awaits additional investigation.

Slater, Ward, and Kunkel [21] have recently shown that myeloma globulins differ from one another, indicating individual specificity. The intriguing concept relating the production of such a broad group of abnormal proteins to wide variations in myeloma cell morphology by these workers implies that individually characteristic proteins may qualify the disease from one person to another. Teilum [25] has studied cases of hyperglobulinemia associated with plasmocytosis and found the presence of paramyloid or amyloid. He suggests that amyloid varies in composition from one case to another and even in different areas within the same case. This would help account for the discrepancies often noted in the tinctorial properties of various amyloids. For this reason, the arbitrary classification of amyloidosis based on localization and staining reactions is difficult

to accept.* The differences in the protein composition of amyloid noted in this paper would agree with the concept that a variety of amyloids may exist.

Amyloidosis has largely been regarded as the connective tissue deposition of an abnormal circulating protein complex. The striking similarity in Case B between the serum protein-polysaccharide fractions and the amyloid protein-polysaccharide fractions seems to confirm this mode of amyloid formation. The abnormally elevated serum lipoprotein in Case B apparently is not significant in the production of amyloid. This abnormal circulating protein-polysaccharide complex has been referred to as paraproteinemia. The anatomical corollary of paraproteinemia is paraproteinosis, a concept advocated by Apitz.[1] This apparent association of connective tissue ground substance alterations with abnormal plasma proteins has led Ehrich [4] to include paramyloidosis with the "collagen diseases."

The various electrophoretic patterns noted with the control tissues (Table 2) are not readily explained but are sharply contrasted when compared with amyloid.

SUMMARY AND CONCLUSIONS

Two cases of amyloidosis have been studied as regards the protein composition of amyloid. Serum protein, lipoprotein, and mucopolysaccharide patterns in one case were evaluated by filter paper electrophoresis and chemical methods. Amyloid from Case A was largely composed of an α_1-globulin and mucopolysaccharide in the same fraction. The analysis of the amyloid from Case B showed α_2- and β-globulins with corresponding mucopolysaccharide components. These results correlated with the elevated serum α_2- and β-globulins. Lipoprotein substances could not be detected in the amyloid material studied. The hypothesis that amyloid represents the tissue localization of a circulating abnormal protein complex is most attractive. The suggestion is made that a broad spectrum of amyloids can exist.

* References 14 and 28.

Dr. Mildred Phillips prepared many liver samples for analysis. Dr. E. Bossak did the electrophoretic analysis of serum C. K., and Dr. E. Greenspan made the globulin pattern determination of the same serum. Dr. A. Gutman supplied the serum (C. K.) for further studies. Drs. Paul Klemperer and C. George Tedeschi were a constant source of stimulation throughout this work.

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News and Comment

ANNOUNCEMENTS

Problems of Aging.—The New York Academy of Medicine, 2 E. 103d St., New York, will sponsor the Twenty-Eighth Annual Graduate Fortnight, Oct. 10-21, on Problems of Aging. The program will include six morning panel meetings, afternoon hospital clinics, twenty evening lectures, four evening panel meetings, and a scientific exhibit.

Evening Lectures and Panel Meetings at 8:30 p.m.

First Week
October

10 **Opening Address**—EDWARD J. DONOVAN, *President, The New York Academy of Medicine*

What Is Aging? *(Ludwig Kast Lecture):* ALBERT LANSING, *Emory University School of Medicine, Atlanta, Ga.*

Psychological Aspects of Aging: ROSS A. MCFARLAND, *Harvard University*

Experimental Prolongation of the Life Span: CLIVE M. MCCAY, *Cornell University, Ithaca, N. Y.*

11 **Arthritis in the Elderly Patient:** JOSEPH J. BUNIM, *National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.*

The Effect of Aging on the Myocardium: WILLIAM DOCK, *State University of New York College of Medicine at New York City, Brooklyn*

Fluid and Electrolyte Problems in Surgery of the Aged: HENRY T. RANDALL, *Memorial Center for Cancer and Allied Diseases*

Relation of Increased Longevity to Neoplasia: C. P. RHOADS, *Memorial Center for Cancer and Allied Diseases*

12 **Some Physiological Aspects of Aging** *(Wesley M. Carpenter Lecture):* N. W. SHOCK, *National Heart Institute, Bethesda, Md.*

Nutritional Problems of Advancing Age: FREDERICK J. STARE, *Harvard University*

Gastrointestinal Hemorrhage in Elderly Patients: THOMAS P. ALMY, *Cornell University Medical College*

13 **Panel Meeting: Cerebrovascular Disease with Aging:** *Chairman:* IRVING S. WRIGHT, *Cornell University Medical College*

14 **Panel Meeting: Problems of Retirement:** *Chairman:* HOWARD REID CRAIG, *Director, The New York Academy of Medicine*

Second Week
October

17 **Nature of the Response to Stress with Aging** *(Harlow Brooks Lecture):* JAMES L. WHITTENBERGER, *Harvard University*

Evaluation and Preparation of the Patient with Degenerative Cardiovascular Disease for Major Surgery: JOHN S. LA DUE, *New York Hospital*

The Aging Lung: DICKINSON W. RICHARDS, *College of Physicians and Surgeons, Columbia University*

October 18 **Management of Injuries in the Aged:** ROBERT H. KENNEDY, *Beekman-Downtown Hospital, New York*

Management of Surgical Emergencies in the Aged: CONDICT W. CUTLER, JR., *Goldwater Memorial Hospital, New York*

Surgery of Chronic Diseases in the Aged: FRANK GLENN, *New York Hospital*

Anesthesia in the Aged: EMANUEL M. PAPPER, *Presbyterian Hospital*

19 **Emotional and Psychiatric Problems of Aging:** FRANCIS J. BRACELAND, *The Institute of Living, Hartford, Conn.*

Neurosurgical Treatment of Parkinsonism: IRVING S. COOPER, *New York University–Bellevue Medical Center*

Rehabilitation in the Aged: HOWARD A. RUSK, *New York University–Bellevue Medical Center*

20 **Panel Meeting—Gonadal Function and Involution in Man—and Related Practical Management:** *Chairman:* EARL T. ENGLE, *College of Physicians and Surgeons, Columbia University*

21 **Panel Meeting—Disorders of the Special Sense Organs Associated with Aging: Their Recognition and Treatment:** *Chairman:* CHARLES F. WILKINSON, JR., *New York University–Bellevue Medical Center*

Morning Panel Meetings—11:00 A. M. to 12:30 P. M.

Monday, Oct. 10

Aging and Atherosclerosis.....*Chairman:* HENRY S. SIMMS

Wednesday, Oct. 12

Everyday Problems in the Care of the Aged.*Chairman:* FREDERIC D. ZEMAN

Friday, Oct. 14

Management of Cardiovascular Renal Problems in the Aged
..... *Chairman:* HUGH LUCKEY

Monday, Oct. 17

Management of Prostatic Obstruction...*Chairman:* ROBERT S. HOTCHKISS

Wednesday, Oct. 19

Public Health Aspects of Aging.....*Chairman:* W. G. SMILLIE

Friday, Oct. 21

Problems of Infectious Diseases and Their Therapy in the Aged
.....*Chairman:* PERRIN H. LONG

All inquiries regarding fees and requests for registration should be addressed to: Secretary, Graduate Fortnight, The New York Academy of Medicine, 2 E. 103d St., New York 29.

Books

Endothelium. By Rudolf Altschul, M.U.Dr., Professor of Histology, University of Saskatchewan. Price, $3.50. Pp. 157, with 10 illustrations. The Macmillan Company, 60 5th Ave., New York 11, 1954.

This small monograph reviews a great deal of material of historical and current interest relating to the ubiquitous cell species lining the vascular tree. This group of cells enters into many important reactions, as its author indicates. The review of the older literature is interesting, insofar as it illustrates the source of current conceptions of the nature of the endothelium. In addition to the historical introduction, Dr. Altschul deals with the morphology, embryology, functions, and pathologic alterations in the endothelium. The section on pathology covers a wide range of injury reactions, including shock, ionizing radiation, hypertension and renal disease, and spontaneous and experimental arteriosclerosis. Unfortunately, the author has cited the opinions of the investigators rather than critically evaluating the capacity of their methods to support their contentions. It becomes quite evident from the presentation that our knowledge of the structure and functions as well as the pathologic alterations occurring in various parts of this ubiquitous system of cells is to this day quite fragmentary and that this is partly due to the limitation of observational techniques. The book serves a very useful and timely purpose in presenting an extensive bibliography of 359 references. It should be of interest to anyone concerned with the pathology and physiology of the vascular system.

Reactions with Drug Therapy. By Harry L. Alexander, M.D. Price, $7.50. Pp. 301, with 33 figures. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1955.

This little book is timely in view of the many problems of drug hypersensitivity incident to the increasing use of chemotherapeutic agents. The author has had long experience in this field, and he has performed an important service in making this information more accessible. The book is well organized and well indexed, and each chapter contains an excellent list of references. The book is well printed and the photographs are of high quality.

The various chapters deal with mechanisms, dermatologic manifestations, systemic patterns, anti-infectious drugs, particularly chemotherapeutic and antibiotic drugs, antiarthritic drugs, sedatives, antihistaminics, organ extracts, vitamins, etc. In assembling the literature dealing with the problems of hypersensitivity, the author points out that "it has been estimated that over 350,000 substances have been employed as drugs." It has also been stated repeatedly "that almost any drug may sensitize man and thereby pave the way for untoward reactions." The author points out, however, that the latter statement is not borne out by the facts, and that, at most, only a few hundred have been recorded as inducing hypersensitivity. Because he has been able to simplify the problem, he has presented a much more precise analysis of the situation. This book should be of great value to all those who are concerned with the problems of hypersensitivity in relation to chemotherapy.

Peripheral Vascular Diseases. By Edgar V. Allen, B.S., M.A., M.D., M.S., F.A.C.P., Professor of Medicine, Mayo Foundation, Graduate School, University of Minnesota; Nelson W. Barker, B.A., M.D., M.S., F.A.C.P.; Edgar A. Hines, B.S., M.A., M.D., M.S., F.A.C.P., and thirteen contributors from the Mayo Clinics. Second Edition. Price, $13.00. Pp. 825, with 316 illustrations, 7 in color. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1955.

This valuable volume has been extensively revised in its second edition. A large amount of material on the anatomy of normal vessels and the fundamental nature of peripheral vascular disease has been deleted and the parts of the book dealing with therapy have been expanded, especially those dealing with recent advances in surgical treatment. The changes in organization should increase its value to those clinicians concerned with the diagnosis and treatment of

peripheral vascular disease. The medical students and investigators who use this monograph will usually have access to other books which will supplement their reading in this volume. They should find the up-to-date bibliography valuable for this purpose.

In this edition the consideration of the following subjects has been extended: peripheral arteriosclerosis and atherosclerosis, hypertensive ischemic ulcers of the leg and purpura, as well as the important advances in surgical treatment of vascular disease which have occurred since the first edition was published.

Pathologists should continue to find this book valuable in adding to their understanding of peripheral vascular disorders. Many of these processes, which are frequently neglected in the general pathological literature, are well described and illustrated, both grossly and microscopically. It may stimulate them to increase their interest in and to investigate more completely these important disease processes of the peripheral vessels which may be omitted in the usual post-mortem examination.

The Skin. By Arthur C. Allen. Price, $25.00. Pp. 1048, with 495 figures. C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1954.

This clinicopathologic treatise on the skin should fill a real need for many pathologists and dermatologists. It represents a complete consideration of the entire spectrum of dermatologic disease, emphasizing the clinical characteristics and the gross and microscopic morphology of each disorder. Brief descriptions of the etiology, pathogenesis, diagnostic criteria, and therapy are included whenever they contribute to an understanding of the disease.

The illustrations of the gross and histological characteristics of the various diseases are beautifully reproduced and grouped. They have been carefully selected and skillfully photographed by the author to illustrate the pertinent points of each disease entity. They appear to represent the more illustrative examples from a wealth of material which the author has been privileged to examine during his association with the New York Skin and Cancer Clinic, the Armed Forces Institute of Pathology, and the Sloan-Kettering Memorial Cancer Center.

To serve the purpose which the author has outlined, that of increasing the interest and understanding of the pathologist in dermatologic disorders, the volume begins with chapters on the embryology, anatomy, and physiology of the skin and the necessary definitions, eponyms, and the regional distribution of the dermatoses.

The dermatoses are given the greatest consideration and the chapters are organized, so far as possible, in an etiological pattern, beginning with the noninfectious dermatoses, those with prominent collagen alterations, the allergic dermatoses, and then the viral, bacterial, mycotic, and protozoal, etc., infestations. Next, the nutritional and vascular abnormalities are presented. Finally, a consideration of the neoplastic diseases of the skin and its appendages makes up the latter quarter of the book.

The bibliography is extensive and up-to-date, and it should also contribute to the value of the volume as a reference book, not only for pathologists and dermatologists, but also for the established general practitioner, the medical student, and the young house-staff member.

It may be predicted that the volume will help reach the author's goals, "to interest his fellow pathologists in the field of cutaneous disease" and to reduce "the barriers between dermatology and other disciplines."

Standard Values in Nutrition and Metabolism. Edited by Errett C. Albritton, M.D. Price, $6.50. Pp. 380, with 160 tables. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1954.

This book was prepared under the direction of the Committee on the Handbook of Biological Data, American Institute of Biological Sciences of the National Research Council. In editing it, Dr. Albritton obtained contributions from several hundred authorities and assembled them in tabular form. Here one will find data concerned with nutrition and metabolism of man and other animals, including nutrient requirements, pathways of metabolism, extensive reference lists, and a complete index. The handbook will be of use to laboratory workers in many fields, particularly in the general field of nutrition and metabolism. According to the editor, "The data in each table are in the judgment of the contributors and reviewers established fact and free of questionable material, and represent the consensus of expert judgment and experience in the special field from which it was drawn."

The Pineal Gland. A Review of the Physiologic Literature. By Julian I. Kitay and Mark D. Altschule. Price, $5.00. Pp. 280, with 8 illustrations. Harvard University Press, Cambridge 38, Mass., 1954.

This is an exhaustive, if unexciting, survey of the world's literature on the pineal gland. The book is short (104 pages of text, 162 pages of bibliography) but is a readable distillation of those investigations and observations which were regarded as statistically significant. From their efforts the authors reached the conclusion that it is not justifiable to regard the pineal gland as vestigial or functionless. A hormonal function was suggested by the potentially secretory parenchymal cells of the pineal gland. The active metabolism of the cells was indicated by histochemical studies, including work with radioactive tracers. The correlation of physiologic experiments involving the removal of the gland and the effects of administering crude pineal extracts with clinical reports of patients with pineal anomalies (predominantly tumors) suggest that the pineal may indeed influence gonadal development, perhaps via the hypophysis. The support for other functions of the pineal gland appear much more tenuous. A new classification of pineal tumors is also suggested. If the authors' purpose in compiling this volume was to stimulate the interest of other investigators in studying the pineal gland, they have probably succeeded.

The Joints of the Extremities. By Raymond W. Lewis, M.D. Price, $8.50. Pp. 104, with 102 illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1955.

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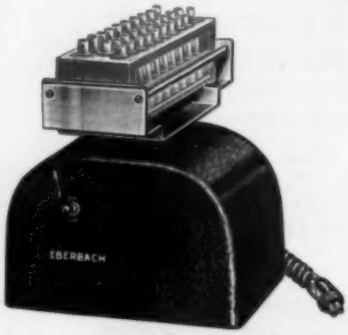

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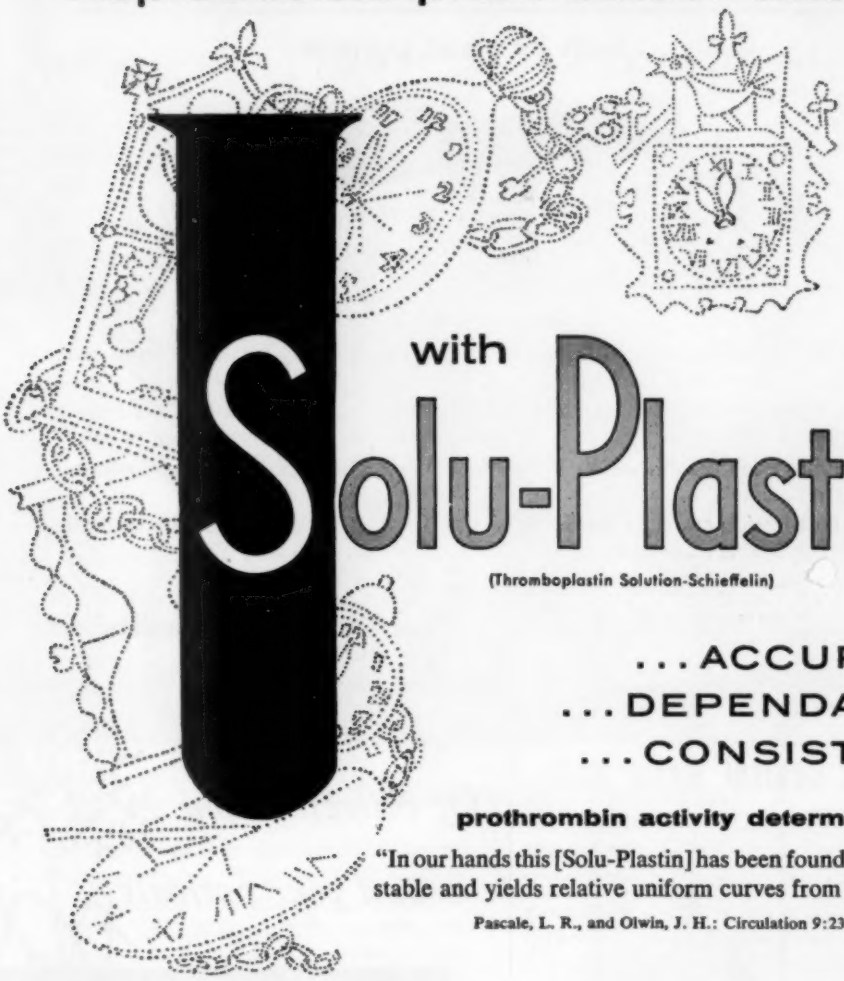

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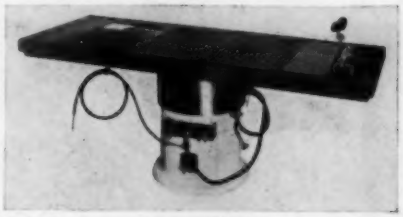

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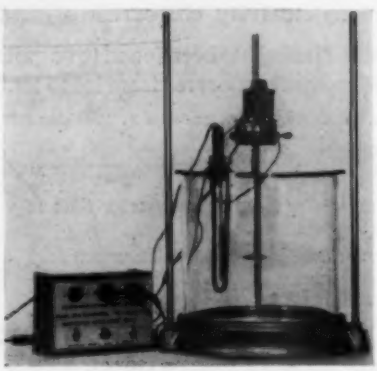

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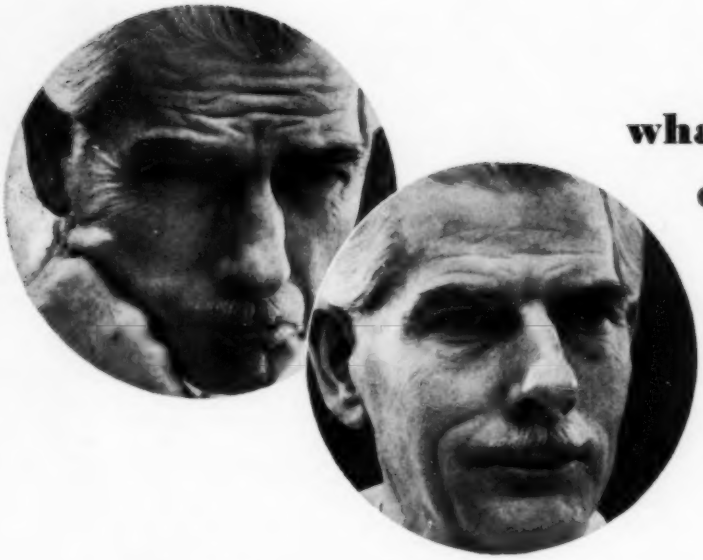

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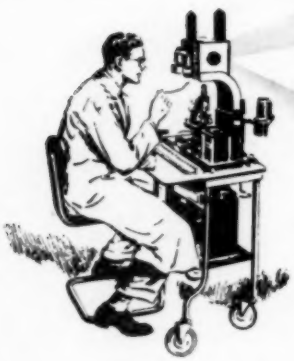

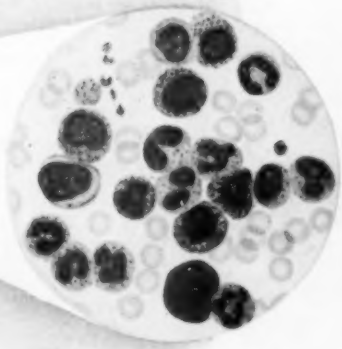

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